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RELATION OF CONFORMATION AND ANATOMY OF THE DAIRY COW TO HER MILK AND BUTTERFAT PRODUCING CAPACITY

UDDER CAPACITY AND MILK SECRETION*

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Although the mammary gland is one of the most important parts of the dairy cow, its internal anatomy, its capacity, and its performance are none too well understood. In much of the literature on dairy type or conformation, comment on these points is for some reason omitted. References which are available are not by any means in close agreement.

Concerning the general structure of the mammary gland Sisson (12) is perhaps most specific, yet he does not comment on its capacity or the milk-secreting process. Plumb (10) discusses udder structure only in a general way and states that no such irregularity of form is presented by any other anatomical part of the domestic animal as by the mammary gland of the dairy cow. Wing (13) gives a general discussion of the glandular make-up. Curtis (3) states that the size of the udder is indicative of capacity for milk production just as the size of the barrel is indicative of the capacity for food consumption. Others, in discussing dairy type, have given a more or less general and superficial description of the mammary gland.

A few have presented more definite statements concerning the capacity of the mammary gland. Isaachsen (7) is specific on this point and, in referring to cows producing 5 to 6 kilos of milk at one milking, says that "no udder contains so much." He continues by stating, "In our animals, the maximum capacity is

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[1] Acknowledgment is made to T. E. Woodward and Dr. F. W. Miller for helpful suggestions and assistance in the conduct of this experiment; and to Dr. L. A. Rogers for determination of milk analyses.

about 3.5 kilos or a little more, and 2 to 2.5 kilos must be formed in these animals at the moment of milking." Marshall (8) refers to calculations showing that "the udders of a cow could not contain the quantity of milk which can be obtained from them at one milking." According to Woods (14), "The flow of milk at the time of milking is usually much greater than the capacity of the milk cistern, but this is readily accounted for, as the irritation of the nerves causes the contraction of the wall of the glands and milk 'ducts." Scott (11) is quoted as follows:

> The reservoir or milk cistern seldom holds more than half a pint of milk. There is only one of these reservoirs in each quarter, so the combined volume of milk in the four cisterns is only two pints. This is the amount of milk which is to be found in the average dairy cow's udder at any one time.
>
> If you could look inside an udder just before milking time you would find the milk cisterns and ducts distended with milk which, as I have already mentioned, would only be about one-half pint in each quarter.

Gaines (5), on the other hand, presents data showing that (*a*) the capacity of the mammary gland of the goat is greater than the volume of milk drawn at one time; (*b*) the udder shrinks in volume during milking to nearly the same extent as the volume of the milk drawn; and (*c*) practically all the milk drawn is present as such in the gland at the beginning of milking.

Those contending that the capacity of the gland is decidedly too small to contain the quantity of milk secured at one milking, assume that milk secretion is greatly accelerated during the act of milking. Many of the exponents of this view claim that nearly all the milk, except approximately one-half pint contained in each cistern, is actually secreted while the milk is being drawn. Gaines (5) and Woods (14), however, are of the opinion that milk secretion is a continuous process. Gaines (5) comments on the work of Eckhard and on that of Goltz and Ewald, which indicates that the mammary gland is not under nervous control. McKenzie (9) also concludes that the mammary gland is not under the direct influence of the nervous system. Woods (14) contends that the manipulation of the teats and udder stimulates the nerves, causing a muscular contraction and expression of the milk. Gaines (5) concludes that nursing or milking excites a reflex contraction of the gland and musculature, with consequent expression of milk.

Regarding the process of milk formation there is some variance in opinion. Bitting (2) outlines a theory supported by many, which is virtually that in the secretion of milk the water serum and salts are separated from the blood, and that a fatty degeneration of the cells lining the alveolar cavities produces the fat globules as the degeneration product and the casein as the undegenerated portion of the cells. He quotes one Smith as follows: "The process of milk secretion may, therefore, be regarded as a process of metabolism of the epithelial cells, which undergo decomposition, and discharge the resulting products into the excretory ducts." Bertkau (1), on the contrary, states that milk formation is a true secretory process and in no manner associated with a total or even partial necrosis of the secreting epithelium. The views of Disselhorst (4) regarding the formation of milk fat are in close agreement.

In spite of references expressing views to the contrary, the belief appears to persist rather generally among teachers and other professional men in dairy cattle and veterinary work that the capacity for storing milk in a cow's udder is not more than a half pint to each quarter. It is held that since the capacity is limited, the quantity of milk produced at any one milking could not be stored or contained in the udder, and that the greater part of the milk is secreted during the process of milking, the rapid acceleration in secretion being the result of a nervous reaction stimulated by the manipulation of the udder and teats.

The distention of the udder just before milking is a common observation. Adherents to the belief that milk secretion takes place chiefly during the act of milking have explained this distention on the theory that immediately before the milk is drawn the udder is gorged with the blood carrying the materials from which the milk constituents are to be made. Both the arterial and venous systems of several udders have been filled after their removal from the body and their total capacity has been found to be very small. The capacity of the circulatory system within the udder therefore could hardly be an important quantitative factor in causing this distention.

The study now being conducted by the writer to determine the relation of the conformation and anatomy of the dairy cow to her milk and butterfat producing capacity has been developed to include an extensive consideration of the mammary gland. Udders are studied comparatively and in relation to their producing abilities as shown by production records. In preparing an udder for one of the phases of the study, formalin is injected into it through the teats with a milking tube attached to a pump. In filling the udder, pumping is continued until it is full and firm. It is undoubtedly distended to a greater extent than it would normally be immediately before milking at the same stage of lactation, but the pumping is stopped before pressure is sufficient to injure the tissue. The force of injection is against gravity, against the pressure of the air within the udder, and requires forcing the fluid into the fine ducts.

It has been shown that opinions differ widely concerning the capacity of the mammary gland of the cow. Udder capacity as herein discussed is understood to mean the storage space within its secretory system. It is determined by injecting formalin through the teats and measuring the quantity retained within the secretory system. In recent tests the quantity of formalin injected into the udder through the teats has been found greatly in excess of the amount it has generally been supposed the udder would hold. Data for five udders are presented in table 1. No. 245 was a hard, fleshy, fibrous udder. The quantity of formaldehyde injected into it, while not measured with precision, was between 3 and 3½ gallons, or approximately 12,000 cc. This udder had been lactating six weeks but had been badly infected. Such a condition supposedly would tend to reduce its storage capacity. No. 221 was a particularly harsh, coarse, and fibrous type of gland and had been lactating twelve weeks. It also had been infected and was secreting only a small quantity. No. 243 had been nonlactating for twelve months. It was of the meaty type but shrunken in size. No. 459 was loose and yielding and

had been lactating three months, following a premature parturition. No. 292 was a reasonably loose and mellow udder from a junior two-year old, forty-three days advanced in lactation. It appears that an udder capacity of from 3 to 5 gallons is not uncommon. For any who might be accustomed to thinking of udder capacity as the quantity of milk produced in two or more milkings, during a period of twenty-four hours, it should be mentioned that capacities herein given refer to single fillings of the udder.

An effort has recently been made to determine whether the milk in a cow's udder is secreted continuously or chiefly during the few minutes required for the milking process. For the first test an eight-year-old Jersey, no. 459, was selected. Her only official production record, made at the age of two years one month, amounted to 10,153 pounds of milk and 536 pounds of butterfat. Her last calving was on January 29, 1926, when twins were born prematurely. Since that time her milk production had been comparatively low. During the seven-day period from April 16 to 22 inclusive, she averaged 13.91 pounds of milk when milked twice daily. Commencing on April 23, she was milked each morning at 10 o'clock. Each subsequent milking, therefore, represented the secretion of twenty-four hours. The average

TABLE 1

Capacity of the secretory system of the mammary gland

NUMBER OF COW	BREED	PORTION OF UDDER FILLED	AMOUNT OF FORMALIN USED	CAPACITY OF SECRETORY SYSTEM	EQUIVALENT IN MILK
			cc	*cc*	*lb.*
245	Holstein	4 quarters	12,000‡	12,000‡	27.26
221	Holstein	4 quarters	13,000	13,000	29.53
243*	Holstein	2 right quarters	4,700	9,400	21.36
459	Jersey	2 right quarters	6,200	12,400	28.17
292†	Holstein	2 right quarters	10,200	20,400	46.35
Average					30.53

* Nonlactating

† Fresh heifer.

‡ Approximate

production on the three successive days was 12.07 pounds. During this three-day period of adjustment and on the morning of April 27, her feed was not altered in kind or quantity. She finished eating each morning at approximately 6:30 o'clock. Until the afternoon of April 26, she was turned out with the herd for water. The quantity drunk was not measured. In order that the intake of both feed and water might be determined, water was offered her in a bucket on the afternoon of April 26 and on the morning of April 27. As she was not accustomed to drinking from a bucket, the water intake for that period may have been less than for previous days. It amounted to 24 pounds, all of which was drunk on the morning of April 27. A decrease in water intake presumably would tend to reduce the milk secretion for that day.

At ten o'clock on the morning of April 27, she was killed and immediately hoisted for bleeding. As soon as bleeding was complete she was lowered to the floor. The udder, together with an area of the skin extending from about 8 inches anterior to the front attachment to about 8 inches posterior to the rear attachment and from thigh to thigh, was then removed in such a manner that the gland tissue was not cut or injured. It was immediately attached to a specially designed iron frame, the position of the udder being adjusted until it was approximately natural for a standing cow. One hour elapsed between the killing of the animal and the completion of adjustment of the udder on the frame.

At eleven o'clock, twenty-five hours after the previous milking, the milk was drawn into a bucket in the usual manner and with approximately the usual ease and rapidity. The quantity of milk obtained from the udder thus severed and suspended was 9.2 pounds. The udder was permitted to hang in the same position for four hours until 3 p.m., when a full pint of milk (1.07 pounds) was drawn with comparative ease. A total of 10.27 pounds, therefore, was drawn from the udder after all body connections had been severed. Since the blood and lymph circulation, the nervous system, and all other body connections were severed before the milk was drawn, there could have been only a remote possibility for milk to be formed during the milking process as a result either of nervous or muscular action stimulated by the udder manipulation. It is obvious, therefore, that more than 85 per cent of the twenty-four-hour milk production of this cow, as based on an average of the three previous days, was secreted and stored within the gland at the time she was slaughtered.

Samples of each of the two milkings immediately preceding death and each of the two portions of the 10.27 pounds of milk drawn after death were preserved for analysis. The analyses appear in table 2.

TABLE 2

Analyses of milk from no. 459

	10 A. M. APRIL 25, 1926—FORTY-EIGHT HOURS BEFORE DEATH	10 A.M. APRIL 26, 1926—TWENTY-FOUR HOURS BEFORE DEATH	11 A. M. APRIL 27, 1926—FIRST POST-MORTEM MILKING	3 P. M. APRIL 27, 1926—SECOND POST-MORTEM MILKING
Quantity (pounds)	11.00	12.10	9.20	1.07
	per cent	*per cent*	*per cent*	*per cent*
Total solids	14.71	14.65	13.88	10.23
Fat	5.75	5.73	4.50	1.60
Solids-not-fat	8.96	8.92	9.38	8.63
Ash	0.832	0.831	0.877	0.939
Casein	2.88	2.81	3.03	2.88
Albumin	0.75	0.77	0.76	0.75
Sugar	4.498	4.509	4.713	4.061

Immediately after the post-mortem milking had been completed, the right half of the udder was filled with formaldehyde and prepared for the study of its gross structure. The left half was removed by an incision just to the left of the median septum and was preserved for histological analysis. The external appearance of the udder before its removal from the cow is shown in figure 1. Figure 2 shows a vertical transverse section through the right rear quarter and illustrates the internal structure of the gland. The tissue is relatively open in structure with comparatively large cisterns. The fact that these conditions were definitely recorded in the observations made through an entire lactation period many months previously lends value to 2500 to 3000 sets of observations that have been made according to the same plan on other living cows.

A second test was conducted on no. 292, a junior two-year-old Holstein, forty-three days advanced in her first lactation period. Since her production was relatively high she was continued on two milkings daily. From the afternoon of July 1 to the morning of July 8, 1926, she averaged 43.36 pounds of milk daily. During this period the average quantity produced at the morning milking was 21.69 pounds. She was milked regularly at 9:30 a.m. and 9:30 p.m., each milking representing the production of twelve hours. The kind and quantity of feed and the time of feeding and watering were maintained as nearly as possible without change.

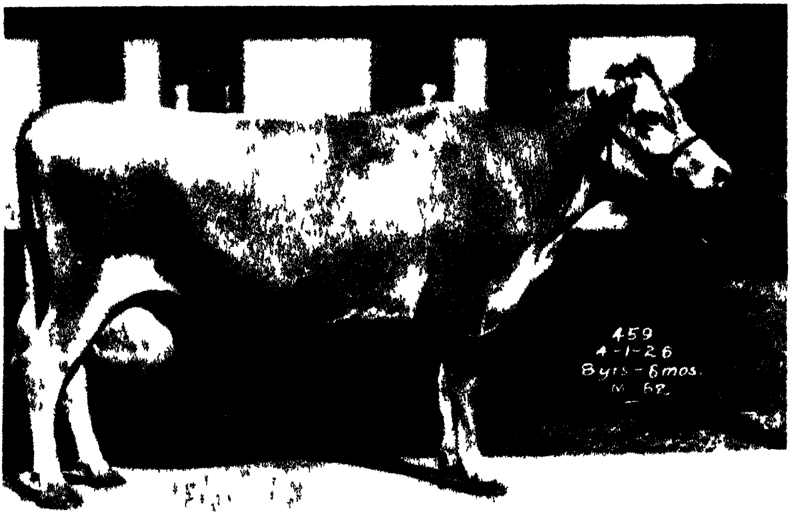

FIG. 1. THE UDDER OF NO. 459 BEFORE ITS REMOVAL

She was killed at 9:30 a.m. July 9, 1926, twelve hours after the last milking. It was necessary to lead her a considerable distance for slaughter and although this was done quietly, she became somewhat excited immediately before death. The effect of this excitement of course is not known. The procedure in bleeding and in removing the udder was identical with that followed in the previous test.

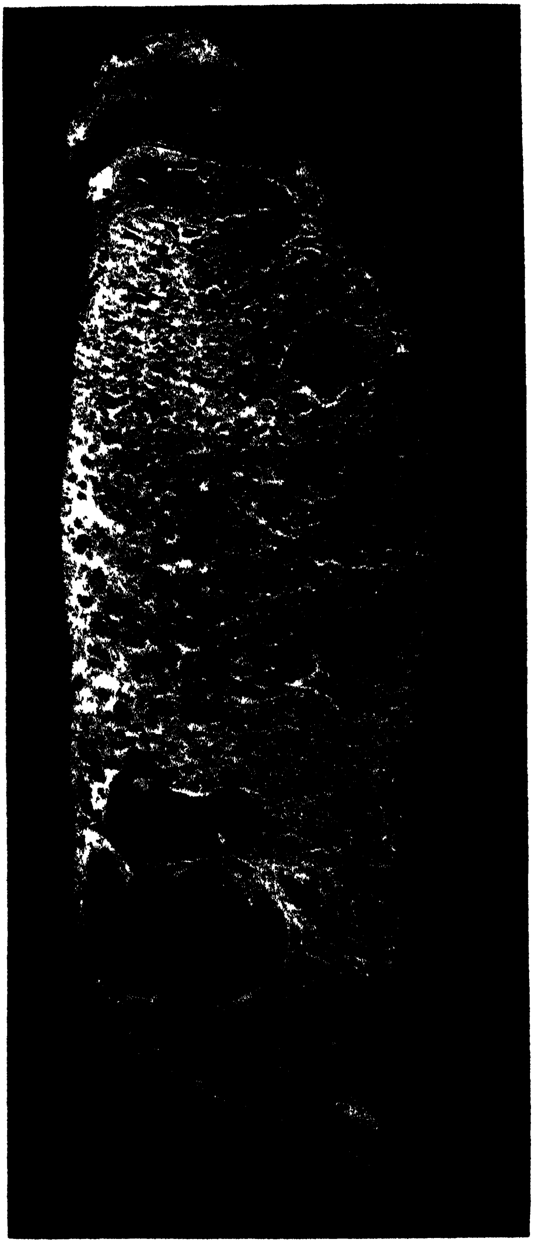

Fig 2. A Vertical Transverse Section through the Right Rear Quarter of No. 459's Udder

Milking was commenced at 10:20 a.m. The milk was drawn much more slowly than in the post-mortem milking of no. 459. The udder was massaged as it was milked. The quantity secured was 7.7 pounds. Four hours later, at 2:20 p.m., the udder was again milked and 2.9 pounds was drawn, making a total of 10.6 pounds after death. When the udder was cut open a short time later the milk gushed from the incision. An abundant quantity of milk appeared to be present in the udder, but it was not so readily drawn as in the previous test. The temperature of the milk was taken at about the middle and near the end of the first post-mortem milking and varied from 96° to 93°F. The temperature of the milk of the second post-mortem milking was 86°F. Samples of the morning milkings of July 7 and July 8 and of both post-mortem milkings were preserved, and their analyses appear in table 3. The udder was treated in the manner described for no. 459. The right half was preserved and sectioned. The external appearance of the udder before its removal from the cow is illustrated in figure 3, and a vertical transverse section through the right rear quarter is shown in figure 4. Reference to table 1 will show the udder capacity of both nos. 459 and 292.

TABLE 3

Analyses of milk from no. 292

	9.30 A. M. JULY 7, 1926—FORTY-EIGHT HOURS BEFORE DEATH	9.30 A. M. JULY 8, 1926—TWENTY-FOUR HOURS BEFORE DEATH	10.20 A. M. JULY 9, 1926—FIRST POST-MORTEM MILKING	2.20 P. M. JULY 9, 1926—SECOND POST-MORTEM MILKING
Quantity (pounds)	24.60	22.00	7.70	2.90
	per cent	*per cent*	*per cent*	*per cent*
Total solids	10.88	10.77	9.61	7.88
Fat	2.95	2.90	1.28	0.70
Solids-not-fat	7.93	7.87	8.33	7.18
Ash	0.757	0.736	0.778	0.818
Casein	2.092	2.021	2.023	1.766
Albumin	0.281	0.313	0.326	0.324
Sugar	4.800	4.800	5.203	4.272

There is a difference between the composition of the ante-mortem and post-mortem milkings which deserves brief comment. This difference is remarkably similar in the milk from both cows. The fourth sample, which is taken from the final post-mortem milking, is the one differing most from the ante-mortem product. In each case the final post-mortem milking shows an increase in ash and a decrease in sugar when compared with that drawn before death. In the case of no. 292, this fourth sample also shows a low casein content. The outstanding difference, however, is in the fat content, which is greatly lowered in both of the post-mortem milkings and largely responsible for the reduction in total solids in these samples.

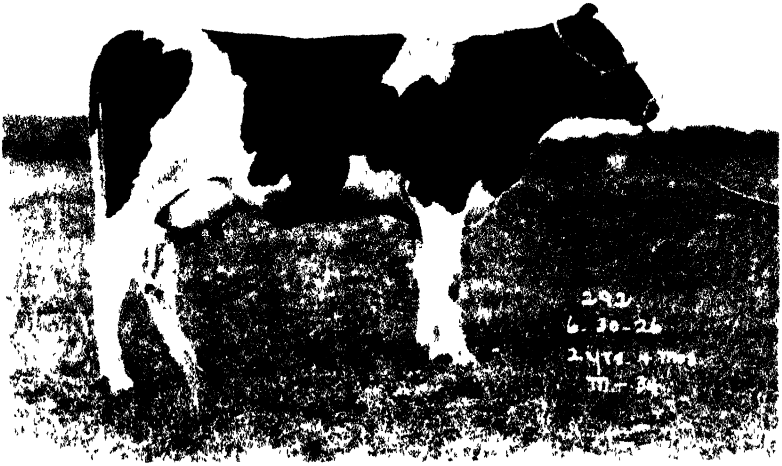

Fig. 3 The Udder of No 292 before Its Removal

If it were true that the fat in milk is a decomposition product of the cells, and if this process actually takes place chiefly during the act of milking, this low fat content might be accounted for. It would seem, however, that if secreted chiefly during the milking of the cow, milk taken from a gland entirely removed from the body and in which all activity apparently had ceased would have a fat content even lower than that found. If the high fat content normally found in strippings and in last milk were due to gravitation, these post-mortem samples, particularly the last one, should be higher in fat content than the others.

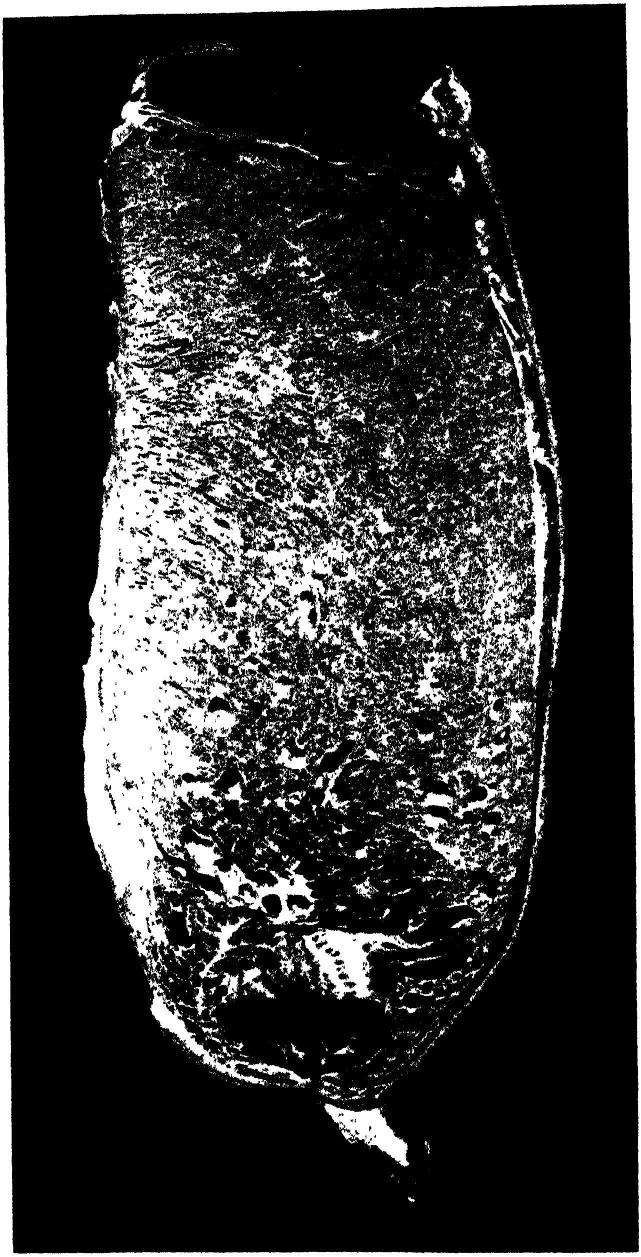

Fig. 4. A Vertical Transverse Section through the Right Rear Quarter of No. 292's Udder

If, on the contrary, milk secretion is a continuous process, with all the constituents secreted simultaneously, the diminished fat content in the post-mortem samples must be accounted for in some other way. Hunziker (6) gives the melting point of mixed butterfat as 90° to 99°F. At lower temperatures the fat presumably would become less fluid. No attempt was made in either case to maintain body temperature in the udder after its removal. The temperature of the post-mortem milk from no. 459 was not determined. For no. 292 it was 96° to 93°F. for the first post-mortem milking and 86°F. for the second. The change in consistency of the fat as a result of the lowered temperature of the gland might cause the retention of a large proportion of the fat in the smaller ducts and easily account for the abnormally low fat content in the post-mortem milkings.

The first two tests reported would indicate that:

1. Milk secretion is to a considerable extent a continuous process.

2. A large proportion of the milk secured at any milking is collected and stored within the gland before the milking process is commenced.

3. The internal capacity of a lactating cow's udder appears to be greater than the volume of the milk secreted. The two udders on which results are here reported were not exceptionally large, yet one of them indicates an internal space sufficient to hold as much as 45 pounds of milk.

4. The liberation of milk is not entirely dependent either upon a nervous or a mechanical stimulation or upon internal muscular contraction, since the act of milking was in both cases performed after all body connections had been severed. While in the post-mortem milking, no. 459 readily liberated about 85 per cent of the amount produced in like periods before death, no. 292 yielded only about 49 per cent. The gushing forth of milk from an incision subsequently made in the udder of no. 292 indicates that in her case the milking was only partially complete. Apparently a relatively high proportion of her average milk production for a like period was present in the udder at the time of death. The reason for the greater difficulty in removing the milk in her case

is not definitely understood. It would seem, however, that muscular contraction, or in this case, *rigor mortis*, may have hindered rather than aided the removal of the milk.

5. The outstanding abnormality in the milk drawn after death is its low fat content. This might be accounted for on the theory that the fat globules were retained within the ducts as a result of the lowered temperature after death of the gland and of the milk contained within it.

The results of these tests are not conclusive. Additional tests will be conducted and the results published as material is made available.

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A STUDY OF THE PROTEOLYTIC ACTION OF CERTAIN SPECIFIC ORGANISMS ON MILK PROTEINS IN MILK AND SYNTHETIC BUTTER*

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Considerable work has already been done by a number of investigators on bacterial metabolism including yeasts and molds, with a view of obtaining a quantitative relation of the action of specific organisms when grown in different media. These investigations were generally limited to pathogenic organisms. However, only in a few instances has milk been used as a media. It is evident that in milk we have a natural product, composed of proteins, carbohydrate and mineral salts, well adapted for bacterial growth, the study of which is of prime importance to the dairy industry. It is well known that the composition of the media influences the growth and development of bacteria. For the study of pathogenic organisms the media is usually composed of nitrogenous materials and an adequate supply of mineral salts. It has been found that when these organisms are grown in a media containing some utilizable carbohydrate, that the chemical changes produced in the media are quite different than when grown in media containing no carbohydrate. Notably, at least in some organisms, no proteolytic enzymes are formed (1) (2) (3).

In our investigation we have confined our study of the proteolytic action of certain specific organisms usually found in milk and manufactured dairy products. This involves the study of bacterial metabolism or cellular activity. The protein requirements and the energy requirements must be supplied by the food material in the media. There is sufficient evidence that the

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cellular activity of all organisms are intimately associated with enzymes, and it is by this means that chemical changes are brought about, which gives us a measure of the rôle played by organisms in the decomposition of the food material in the media.

The proteolytic enzymes of organisms may be classified into two well defined groups—the peptic group of enzymes which act and are accelerated in acid media; the tryptic group which act and are accelerated in an alkalin media. Some organisms secrete both groups. In fact, no enzyme has yet been found or isolated which is specific in action as a unit rather than a mixture. This fact of a mixture of the two groups of enzymes is unmistakably shown in our previous work on the enzymes of B. panis migula (5). In this investitation it was found that when the enzyme acted in a 0.2 per cent acid solution, 5.1 per cent of the total nitrogen apeared as amino acids; when acting in a 0.2 per cent basic solution 14.00 per cent of amino acids was formed both solutions being digested for thirty-three days. However, when digested in neutral solution, 25.35 per cent of amino acids was formed. The characteristic action of both the tryptic and peptic enzymes was clearly manifested in this experiment (5).

In addition to the proteolytic enzymes, there is usually accompanied sugar splitting enzymes as well as fat splitting enzymes. In this investigation we are concerned only with the protein splitting enzymes. In dairy products we have both the protein and sugar as lactose. As lactose is hydrolyzed by enzymes of a zymase character, the acid produced will influence to a great degree the soluble or exo enzyme production of the organism (1), (2), (3), and consequently proteolytic action is retarded. There is a maximum degree of acidity for the growth and development of the organisms themselves. Unless soluble proteolytic enzymes are formed during the process of bacterial growth, no proteolytic action will take place even though the media is neutralized. In our study of B. panis migula when grown in sterile milk we found at the end of thirty days 26.40 per cent of total nitrogen appearing as amino acids. Fifteen days longer incubation brought about no increase neither in ammonia nor amino acids, the acidity of the milk being at the end of 45 days, 0.540 per cent. The high acidity inhibited the action of the enzymes and the functioning of the organism.

The production of ammonia by means of bacterial action is a measure of bacterial metabolism, taking place within the organism. In the utilization of protein, the molecules are hydrolized to simpler compounds, peptones, amino acids, etc. When these compounds are utilized for energy production, deaminization takes place and the ammonia is the end product and appears in the media. The production of ammonia is no indication of the changes taking place exterior to the organism. It is therefore evident, to gain a more definite idea and interpretation of protein decomposition, it is necessary to secure data of the amino acids, ammonia and peptones produced by proteolytic organisms.

The determination of titratable acidity is an indirect measurement of the dissociated acidity or hydrogen-ion concentration, if this is based on a given acid and for a media of constant composition.

In this study of the proteolytic action of organisms we have limited our data to what seemed to us the most important cleavage products and to show to what extent, under certain conditions, these changes have taken place in proteins of milk and synthetic butter. Owing to the separation of the proteins during sterilization it was found impossible to sterilize butter, and bring it to the original homogeneous condition.

It is known that it is difficult to account for the numerous changes caused by the organisms; nor is it possible to solve complex problems with the sum total action of these organisms. If we can trace the deterioration of groups of organisms, some good has been accomplished. It seemed therefore desirable to eliminate this complexity and use specific organism in sterile media.

BACTERIOLOGICAL WORK

Milk. Two hundred fifty cubic centimeters of fresh skimmilk were sterilized in a 500 cc. Erlenmeyer flask in an autoclave at 15 pounds for thirty minutes. In the sterile milk is inoculated 1 cc.

of a twenty-four-hour culture of the organism to be studied. Incubation temperature was room temperature (20° to 21°C.) Analyses were made each fifteen days.

Synthetic butter. Six hundred-gram lots of synthetic butter were prepared as follows: Pure butterfat was obtained by melting creamery butter at a temperature between 60° to 70°C. When the water and curd were well separated by gravity the clear fat was filtered and sterilized in definite amounts. Fifty grams of skimmilk with an acid reaction of 0.17 per cent were introduced into a 500 cc. Erlenmeyer flask and sterilized in an autoclave at 15 pounds pressure for thirty minutes. To this sterile skimmilk a pure twenty-four-hour culture of the organism to be studied was introduced and incubated for twenty-four hours at 37°C. The sterile fat, was introduced into the inoculated skimmilk. The flask was gradually cooled in ice water, and gently shaken so as to insure a homogeneous consistency. The blank was prepared in like manner except that it was not inoculated. The synthetic butter had approximately the following composition: protein 0.6 per cent, water 14.6 per cent, lactose 1.0 per cent, and fat 84.0 per cent. The synthetic butter was held at room temperature and at 0° to 4°C. The synthetic butter held at room temperature was submitted to analysis each 15 days while that at 0° to 4°C. was analyzed every thirty days.

The organisms used were obtained from reliable sources and rechecked in order to determine their purity and nomenclature. Twenty-four-hour cultures were made into sterile skimmilk for three successive days in order to vitalize the organism. The following organisms were used:

Bacillus panis
Pseudonionas viscosa
Proteus vulgaris Hauser
Bacillus megatherium
Bacillus amylobacter
Bacillus mesentericus Flugge
Streptococcus lactis Lester
Escherichia coli
Bacillus mycoides
Achromobacter liquefaciens
Bacillus subtilis
Aerobacter aerogenes
Escherichia ichthyosmia

The above nomenclature is according to Bergey's Manual of Determinative Bacteriology.

CHEMICAL WORK

The chemical work in this investigation consisted in determining the titratable acidity, nitrogen compounds not precipitated by phosphotungstic acid and nitrogen compounds not precipitated in a saturated solution of zinc sulphate. The peptones represent the difference between the nitrogen compounds not precipitated by zinc sulphate and phosphotungstic acid. It is generally agreed that phosphotungstic acid precipitates all the higher complexes including the diamino acids, but does not precipitate the mon-amino acids. The nitrogen compounds not precipitated by phosphotungstic acid are frequently considered as representing only amino acids, which should be accepted as relatively correct. We have found in previous work[1] the amino acids as determined by the Van Slyke method are lower, but with practically a constant difference. The data represent more correctly, representative well defined groups of protein compounds and show the progress of protein hydrolysis.

The ammonia was determined by the Folin aeration method. Two hundred cubic centimeters of the milk was used and after aeration this was neutralized and used for the following determinations. To 10 cc. of the aerated milk sufficient 20 per cent sulphuric acid was added to make the acidity 5 per cent. To this a 5 per cent solution of phosphotungstic acid solution was added to cause complete precipitation of the proteins. It was then set aside at room temperature for twelve hours, then filtered, and the nitrogen determined in the filtrate by using the Kjeldahl method.

To 10 cc. of the aerated milk dilute sulphuric acid was added to give a distinct acid reaction to methyl red. This was saturated with pure zinc sulphate by slightly warming and left standing at room temperature for twelve hours. This was then brought up to 30 cc. by adding a saturated solution of zinc sulphate. This was filtered and 15 cc. of the filtrate was used for the nitrogen determination. The peptones were estimated by subtracting the nitrogen not precipitated by phosphotungstic acid from the nitrogen not precipitated by a saturated solution of zinc sulphate. The results in tables were based on the total nitrogen.

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[1] Unpublished data.

Two hundred and fifty grams of butter were weighed into a beaker, warmed and transferred to a 500 cc. flask. About 100 cc. portions of water at 50° to 60°C. was poured into the beaker, and the mixture transferred to the flask after standing to permit

TABLE 1

Acidity produced by the respective specific organisms inoculated in sterile milk held at 20°C.

	15 DAYS INCUBATION	30 DAYS INCUBATION	45 DAYS INCUBATION	CONTROL
	per cent	*per cent*	*per cent*	*per cent*
panis	0.324	0.409	0.450	0.243
viscosa	0.170	0.103	0.108	
vulgaris	0.170	0.090	0.108	
megatherium	0.464	0.504	0.414	
amylobacter	0.310	0.378	0.378	
coli	0.67	0.77	0.78	
mesentericus	0.74	0.31	0.56	
lactis	0.925	0.70	0.91	
liquefaciens	0.396	0.740	0.675	
mycoides	0.19	0.49	1.09	
subtilis	0.378	0.387	0.540	
aerogenes	0.634	0.693	0.727	
ichthyosma	0.531	0.414	0.414	

TABLE 2

Ammonia produced by the specific organisms inoculated in sterile milk held at 20°C.

	15 DAYS INCUBATION	30 DAYS INCUBATION	45 DAYS INCUBATION	CHECK
	per cent	*per cent*	*per cent*	*per cent*
panis	0.50	1.29	3.91	0.32
viscosa	0.30	0.51	1.25	
vulgaris	0.42	0.51	1.14	
megatherium	1.84	4.13	11.65	
amylobacter	1.47	2.61	5.40	
coli	0.10	0.13	0.97	
mesentericus	0.30	1.88	4.05	0.34
lactis	0.13	0.24	0.38	
liquefaciens	4.32	7.01	7.69	
mycoides	0.73	1.43	3.96	
subtilis	2.42	5.67	7.27	0.35
aerogenes	1.88		2.57	
ichthyosma	9.03	20.16	25.68	

the separation of the aqueous portion from the fat; the separated water and proteins were pipetted into a 250 cc. graduated flask.

TABLE 3

Nitrogen compounds not precipitated by phosphotungstic acid produced by the respective organisms inoculated in sterile milk held at 20°C.

	15 DAYS INCUBATION	30 DAYS INCUBATION	45 DAYS INCUBATION	CHECK
	per cent	*per cent*	*per cent*	*per cent*
panis	7.10	8.93	17.00	3.47
viscosa	4.04	4.26	4.49	
vulgaris	3.50	3.52	8.74	
megatherium	12.18	12.56	27.66	
amylobacter	7.51	12.11	18.83	
coli	3.75	3.61	4.06	3.35
mesentericus	8.65	13.65	20.00	5.00
lactis	4.40	5.33		5.15
liquefaciens	16.14	31.63	31.86	4.93
mycoides	5.38	9.42	19.99	4.93
subtilis	8.61	17.57	31.13	3.41
aerogenes	3.01	3.87	8.32	3.42
ichthyosma	33.20	51.90	53.01	3.02

TABLE 4

Nitrogen compounds not precipitated by a saturated solution of zinc sulphate produced by the respective organisms inoculated in sterile milk held at 20°C.

	15 DAYS INCUBATION	30 DAYS INCUBATION	45 DAYS INCUBATION	CHECK
	per cent	*per cent*	*per cent*	*per cent*
panis	13.46	15.38	26.92	4.60
viscosa	6.51	6.56	6.75	4.60
vulgaris	4.60		8.97	4.60
megatherium	16.37	15.00	33.62	4.60
amylobacter	11.66	18.84	29.16	4.60
coli	5.86	6.76	6.31	4.60
mesentericus	17.30	31.87	41.75	7.28
lactis	6.53	8.40		7.28
liquefaciens	22.43	38.70	50.25	7.28
mycoides	10.77	22.43	39.04	7.28
subtilis	22.36	22.36	32.58	6.55
aerogenes	6.37		10.01	6.55
ichthyosma	66.40	68.30	71.61	6.55

The process of adding warm water to the butter was repeated until 250 cc. of protein solution had been obtained. This process

TABLE 5

Nitrogen compounds as peptones produced by the respective organisms inoculated in sterile milk held at 20°C.

	15 DAYS INCUBATION	30 DAYS INCUBATION	45 DAYS INCUBATION	CHECK
	per cent	*per cent*	*per cent*	*per cent*
panis	6.36	6.45	9.92	1.13
viscosa	2.47	2.30	2.26	
vulgaris	1.10		2.03	
megatherium	4.19	5.44	5.96	
amylobacter	4.15	6.73	10.33	
coli	2.11	3.15	2.25	
mesentericus	8.65	18.22	21.75	
lactis	2.13	3.07		
liquefaciens	6.29	7.07	18.49	
mycoides	5.39	13.01	19.05	
subtilis	13.75	4.79	1.45	
aerogenes	3.36		1.69	
ichthyosma	33.20	16.40	18.60	

TABLE 6

Summary of tables 1 to 5 showing the increase of ammonia, nitrogen compounds not precipitated by phosphotungstic acid and peptones in milk by the action of specific organisms during a period of 45 days based on the total nitrogen

	AMMONIA	NITROGEN NOT PRECIPITATED BY PHOSPHOTUNGSTIC ACID	PEPTONE
	per cent	*per cent*	*per cent*
panis	3.59	13.53	8.79
viscosa	0.93	1.02	1.13
vulgaris	0.82	5.27	0.90
megatherium	11.33	24.19	4.83
amylobacter	5.08	15.36	9.20
coli	0.65	0.71	1.12
mesentericus	3.71	15.00	20.62
lactis	0.06	0.18	1.94
liquefaciens	7.37	26.93	17.36
mycoides	3.64	15.06	17.92
subtilis	6.95	27.72	1.32
aerogenes	2.25	4.90	0.56
ichthyosma	25.36	49.99	17.47

removed more than 96 per cent of the protein. The graduated flask was set aside in a warm place until the particles of fat had come into the neck. These were removed by means of a pipette. This solution was then treated in the same manner as the milk was treated for the determination of ammonia, amino acids, peptones, etc.; in the precipitation by phosphotungstic acid and zinc sulphate, 50 cc. of the protein solution was used.

TABLE 7

Acidity produced by the respective specific organisms inoculated in sterile butter held at room temperature, 20°C.

	15 DAYS HELD	30 DAYS HELD	40 DAYS HELD	60 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	0.033	0.030	0.090	0.091	0.011
viscosa	0.025	0.032	0.198	0.201	0.011
vulgaris	0.015	0.022	0.045	0.049	0.011
megatherium	0.22	0.023	0.018	0.022	0.011
butyricus	0.013	0.011	0.162	0.185	0.011
coli	0.100	0.144	0.148	0.138	0.011
mesentericus	0.067	0.09	0.251	0.248	0.017
lactis	0.175	0.268	0.315	0.340	0.016
liquefaciens	0.109	0.126	0.121	0.101	0.016
mycoides	0.099	0.122	0.125		0.016

TABLE 8

Ammonia produced by the respective specific organisms inoculated in sterile butter held at 20°C.

	15 DAYS HELD	30 DAYS HELD	45 DAYS HELD	60 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis		0.90	1.08	1.43	0.35
viscosa	0.63	1.07	2.14		0.35
vulgaris	0.65	0.79	1.00	1.04	0.35
megatherium	1.11	1.43	1.69	1.78	0.35
butyricus	1.53	2.14	2.14		0.35
coli	0.27		0.71		0.21
mesentericus	1.18		0.80	0.84	0.21
lactis	0.20		0.89	0.87	0.21
liquefaciens	1.25		1.78	2.35	0.21
mycoides	1.07			1.34	0.21

All determinations were made in duplicate.

Analyses of the inoculated milk, synthetic butter held at room temperature and synthetic butter held in cold storage at 0° to

4°C. were made at regular intervals. The milk and butter held at room temperature were analyzed every fifteen days, the butter held in cold storage every thirty days.

TABLE 9

Nitrogen compounds not precipitated by phosphotungstic acid produced by the respective specific organisms inoculated in sterile butter held at 20°C.

	15 DAYS HELD	30 DAYS HELD	45 DAYS HELD	60 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	10.70	12.50	14 29	29.11	4.35
viscosa	7.05	7.50	11 61	20 80	
vulgaris	4 10	5.35	6.25	15.62	
megatherium	16.05	17.94	18 75	23 48	
butyricus	8 75	14 37	14.50	24.10	
coli	3 66	4.73	11 52	11.50	
mesentericus	11.78	11 70	17.68	20 18	
lactis*	3.75	6 43			
liquefaciens	7 96	9.64	12.86	20 44	
mycoides	9.03	8.80	9 18	15 44	

* Contaminated.

TABLE 10

Nitrogen compounds not precipitated by saturated solution of zinc sulphate produced by the respective specific organisms inoculated in sterile butter held at 20°C.

	15 DAYS HELD	30 DAYS HELD	45 DAYS HELD	60 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	20 53	18.75	25.80	50.00	7 58
viscosa	8.75	9.10	12.50	41.07	
vulgaris	6.61	6.70	9.82	38.21	
megatherium	31.25	32.14		41.07	
butyricus	10.00	18.75	18 00	26.24	
coli	4.46	6.00		17.49	
mesentericus	19.01	19.64	24.11	29.37	
lactis	5.80	9 64			
liquefaciens	18.30	20.00	21.25	25.70	
mycoides	16.96		18.60	27.32	

In order to render the results of this investigation of value, extra effort was made to eliminate factors interfering with the uniformity such as temperature of storage and precipitation of proteins. This gives some assurance of the relative comparableness of our results.

Tables 1 to 15 show the changes caused by the organisms studied, in sterile milk, sterile synthetic butter held at room temperature and in cold storage.

TABLE 11

Nitrogen compounds as peptones produced by the respective specific organisms inoculated in sterile butter held at 20°C.

	15 DAYS HELD	30 DAYS HELD	45 DAYS HELD	60 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	9.83	6.25	11.55	20.89	2.23
viscosa	1.70	1.60	0.89	20.27	
vulgaris	2.51	1.35	3.57	12.59	
megatherium	15.20	14.20		17.59	
butyricus	1.25	4.38	3.50	2.14	
coli	0.80	1.27		5.99	
mesentericus	7.23	8.94	7.45	9.19	
lactis	2.05	2.21		2.31	
liquefaciens	10.34	10.36	8.37	5.26	
mycoides	7.93		9.22	11.88	

TABLE 12

Summary of tables 7 to 11 showing the increase of ammonia nitrogen compounds not precipitated by phosphotungstic acid and peptones by the action of specific organisms in synthetic butter held at room temperature for 60 days

	AMMONIA	NITROGEN NOT PRECIPITATED BY PHOSPHO-TUNGSTIC ACID	PEPTONES
panis	1.08	13.76	18.66
viscosa	1.79	14.45	18.04
vulgaris	0.69	10.27	10.36
megatherium	1.43	18.13	15.36
butyricus	1.79	18.75	0.09
coli	0.50	6.15	3.76
mesentericus	0.59	14.83	6.96
acidi lactis	0.66	6.35	0.08
liquefaciens	2.14	15.09	3.03
mycoides	1.13	10.09	9.65

Throughout the tables, only the specific names of organism will be used.

Comments of results from data in tables 1 to 6. The production of acidity as determined by titration of most organisms reached the maximum acidity during the first period of fifteen days.

Panis, mycoides, subtilis, and aerogenes continued to increase the acidity until the end of the third period of forty-five days. This increase was very slight during the second to the third period; evidently the acidity reached its maximum at the end of thirty days incubation.

TABLE 13

Acidity produced by the respective specific organisms inoculated in sterile butter held in cold storage at 0° to 4°C.

	30 DAYS HELD	60 DAYS HELD	90 DAYS HELD	120 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	0.018	0.019	0.020	0.016	0.018
viscosis	0.027	0.030	0.026	0.049	
vulgaris	0.013	0.014	0.013	0.016	
megatherium	0.009	0.011	0.012	0.015	
butyricus	0.004	0.008	0.010	0.017	
coli	0.068	0.117	0.125	0.135	0.036
mesentericus	0.072	0.108	0.112	0.099	
lactis	0.108	0.207	0.224	0.244	
liquefaciens	0.072	0.145	0.144	0.140	
mycoides	0.063	0.126	0.185	0.135	

TABLE 14

Ammonia produced by the respective specific organisms inoculated in sterile butter held in cold storage at 0° to 4°C.

	30 DAYS STORAGE	60 DAYS STORAGE	90 DAYS STORAGE	120 DAYS STORAGE	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	0.252	0.193	0.320	1.34	0.192
viscosa	0.255	0.546		1.01	
vulgaris	0.42	0.319	0.757	0.757	
megatherium	0.377	0.386	0.300	0.840	
butyricus	0.184	0.512	0.462	1.17	
coli	0.420	0.580		0.588	
mesentericus	0.42	0.330	0.625	0.714	
lactis	0.310	0.170	0.312	0.357	
liquefaciens	0.425	1.010	1.00	0.89	
mycoides	0.358	1.258	1.331	1.348	

In the summary of table 6 the production of ammonia, amino acids and peptones is strikingly shown when the organisms are grown in milk as the media. All organisms producing a high per cent of ammonia were likewise active in producing amino acids and peptones, there being some exceptions to the proportion of ammonia, amino acids and peptone. The highest per cent of ammonia produced was that by ichthyosma. Likewise this organism produced the highest per cent of amino acids but not of peptones.

TABLE 15

Nitrogen compounds not precipitated by pho photungstic acid produced by the respective specific organisms inoculated in sterile butter held in cold storage at 0° to 4°C.

	30 DAYS STORAGE	60 DAYS STORAGE	90 DAYS STORAGE	120 DAYS STORAGE	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	6 64	11 01	13 301	27 40	6 64
viscosa	7 14	6 64	8.82	19 57	
vulgaris	4 20	5 40	11.67	23 10	
megatherium	9 40	11 03	13 03	13.70	
butyricus		7.56	10 42	14.30	
coli	3.20	4.46	5 00	5 16	5 00
mesentericus	5.89	8.04	8.93	9 82	
lactis	5 35	4.50	5 80	6 24	
liquefaciens	7 50	9 28	9 47	10 71	
mycoides	6 43	9.19	9 46	9 00	

TABLE 16

Nitrogen compounds not precipitated by saturated solution of zinc sulphate by the action of the respective specific organisms inoculated in sterile butter held in cold storage at 0° to 4°C.

	30 DAYS STORAGE	60 DAYS STORAGE	90 DAYS STORAGE	120 DAYS STORAGE	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	11.76	21 43	23.20	47.07	7 06
viscosis	8 41	8 57	9 91	30.75	
vulgaris	8.40	9.41	13.76	35.98	
megatherium	11.76	12.77	15.30	39.15	
butyricus		14 45	13.61	17.14	
coli	8 30	11.25	11.61	12.32	
mesentericus	9.20	14.82	15.35	16.07	
lactis	9.20	10.35	11.61	12.85	
liquefaciens	11.60	12.60	15.17	16.07	
mycoides	9.55	11.07	11.60	12.05	

Comments of results from data in tables 7 to 12, organisms in synthetic butter held at room temperature and in tables 13 to 18, synthetic butter held in cold storage, time of holding sixty and one hundred and twenty days, respectively. In the synthetic butter there was a continued increase in the acidity of all organisms studied. S. lactis produced an acidity of 0.34 per cent during

TABLE 17

Nitrogen compounds as peptones produced by the respective specific organisms inoculated in sterile butter held in cold storage at 0° to 4°C.

	30 DAYS STORAGE	60 DAYS STORAGE	90 DAYS STORAGE	120 DAYS STORAGE	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	5.12	10.42	9.59	19.67	0.42
viscosis	1.27	1.93	1.09	11.18	
vulgaris	4.20	4.01	2.09	12.88	
megatherium	2.36	1.74	2.27	25.45	
butyricus		6.89	3.19	2.84	
coli	5.10	6.79	6.61	7.15	
mesentericus	3.31	6.78	6.42	6.25	
lactis	3.85	5.85	5.81	6.61	
liquefaciens	4.10	3.32	5.70	5.14	
mycoides	3.12	1.88	2.40	4.05	

TABLE 18

Summary of tables 11 to 15 showing the increase of ammonia, nitrogen, compounds not precipitated by phosphotungstic acid and peptones by the action of specific organisms in synthetic butter held in cold storage for 120 days

	AMMONIA	NITROGEN NOT PRECIPITATED BY PHOSPHOTUNGSTIC ACID	PEPTONES
	per cent	*per cent*	*per cent*
panis	1.148	20.76	19.25
viscosa	0.818	12.93	10.76
vulgaris	0.565	16.46	12.46
megatherium	0.648	7.06	25.03
butyricus	0.978	7.66	2.42
coli	0.396	0.17	6.73
mesentericus	0.523	4.82	5.83
lactis	0.165	1.24	6.19
liquefaciens	0.698	5.71	1.72
mycoides	1.156	4.00	2.63

storage of sixty days at 20°C., table 7. In the synthetic butter held at 0° to 4°F. for a period of one hundred and twenty days,

TABLE 19

A comparison of the actions of proteolytic organisms on the proteins in milk at room temperature, synthetic butter held at room temperature, 20°C. and synthetic butter held in cold storage 0° to 4°C.

ORGANISMS	NITROGEN NOT PRECIPITATED BY PHOSPHOTUNGSTIC ACID			NITROGEN AS PEPTONES		
	Milk	Synthetic butter		Milk	Synthetic butter	
	Room temperature	Room temperature	Cold storage	Room temperature	Room temperature	Cold storage
	At end of 45 days	At end of 60 days	At end of 120 days	At end of 45 days	At end of 60 days	At end of 120 days
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
anis	13.53	13.76	20.76	8.79	18.66	19.25
ulgaris	5.27	10.27	12.93	0.90	10.36	10.76
egatherium	24.19	18.13	7.66	4.83	15.36	25.03
esentericus	15.00	14.83	4.82	20.62	6.96	5.83
quefaciens	26.93	15.09	5.71	17.36	3.03	1.72
ycoides	15.06	10.09	4.00	17.92	9.65	2.63
Average	16.65	12.02	9.30	11.70	10.06	10.90

TABLE 20

Acidity of the inoculated milk and synthetic butters at the end of the respective periods of incubation

Tabulated for the purpose of comparing the acidity produced by these proteolytic organisms with results as shown in table 19

ORGANISMS	MILK	SYNTHETIC BUTTER	
	Room temperature	Room temperature	Cold storage
	per cent	*per cent*	*per cent*
panis	0.450	0.091	0.016
vulgaris	0.108	0.049	0.016
megatherium	0.414	0.022	0.015
mesentericus	0.560	0.248	0.099
liquefaciens	0.675	0.101	0.140
mycoides	0.540	0.125	0.135
Average	0.458	0.109	0.07

S. lactis produced an acidity of 0.244 per cent. The same relative increase in acidity followed when the butter was stored at room temperature for sixty days as when stored at 0° to 4°C. except for B. panis migula, Proteus vulgaris and B. megatherium which produced no acidity as shown by the control.

The production of ammonia by the respective organisms is shown in tables 8 and 14. In each instance there was a gradual increase of ammonia by the organisms whether butter was held at room temperature or in cold storage and this quite uniformly, there being few exceptions. It is to be noted that B. panis migula and B. mycoides produced as much ammonia when butter was held for sixty days at room temperature as when held at 0° to 4°C. for one hundred and twenty days.

That there was a continual increase of both the amino acids and peptones during storage is clearly shown in tables 9 and 11 for butter stored at room temperature, and in tables 15 and 17 for butter stored at 0° to 4°C., there being few exceptions. It is especially to be noted that organisms active in ammonia productions were also correspondingly active in hydrolizing the proteins to amino acids and peptones.

In this investigation the hydrolysis of milk proteins in milk and synthetic butters into simple compounds during a period of forty-five, sixty and one hundred and twenty days is shown in table 19. The organisms are selected for the reason that they were used in both the milk and synthetic butters.

DISCUSSION

In the study of the action of proteolytic organisms we are concerned with end and cleavage products formed. The composition of the media at the beginning and the chemical changes produced during the investigation furnishes evidence as to the character and progress of the changes brought about by organisms used.

It is to be noted that all organisms producing ammonia hydrolized a corresponding amount of proteins into amino acids and peptones. While there is considerable variation in the ratio of these cleavage products for different organisms, this variation may be accounted for if we consider the proteolytic action of enzymes. In our comparison of the changes brought about by six proteolytic organisms during the respective periods of holding, we would, according to the law of mass action, expect a much greater per cent of protein in milk hydrolized than in the synthetic butter, there being in milk 3 per cent protein and in the synthetic butter, 0.6 per cent. Several important factors are involved in explanation of this difference. It has been shown by Sears, Jones and Kendall and co-workers that in presence of a utilizable carbohydrate, as dextrose, at least some bacteria do not secrete proteolytic enzymes. In the hydrolysis of lactose we have both dextrose and galactose which may in a great measure account for the retarded proteolysis of the proteins in the milk. The milk contains approximately 5 per cent lactose while the synthetic butter contains 1 per cent.

The other factor, the acidity of the media seems more evident in influencing the proteolytic changes. For the six organisms the average acidity of milk was 0.458 per cent at the end of forty-five days, at room temperature; that of synthetic butter 0.109 per cent at the end of sixty days at room temperature, and that of synthetic butter 0.07 per cent at the end of one hundred and twenty days at 0° to 4°C. The average per cent of acidity of the milk and synthetic butter at room temperature is approximately proportional to the concentration of the lactose. The high acidity in milk unquestionably influenced the normal development of the organisms as well as the activity of the enzymes.

The proteolytic enzymes formed by bacteria may be either tryptic or peptic or both, the action of the enzymes on proteins producing amino acids in addition to peptones, suggests the presence of tryptic enzymes. There is no evidence as yet to show that each group has an optimum activity at a certain degree of acidity or alkalinity, nor that all proteolytic organisms secrete enzymes of the same character.

In our study of B. panis migula (5), we found that when the acidity reached 0.45 per cent, both the production of acidity and proteolytic action ceased. The preliminary work suggests the possibility and fruitfulness of a more extended investigation of specific organisms and their enzymes. The influence of the hydrogen-ion concentration on bacterial enzymes, the presence or absence of carbohydrates have not as yet been studied to warrant an *a priori* prediction.

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VARIATIONS IN THE SUSCEPTIBILITY OF THE FAT IN DRY WHOLE MILKS TO OXIDATION WHEN STORED AT VARIOUS TEMPERATURES AND IN VARIOUS ATMOSPHERES*

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Practical experience has shown that there is no great improvement in keeping quality of stored butters unless temperatures below 0°C. are used. Relatively low temperatures are therefore employed even when the period of storage is short.

The idea seems prevalent that in the case of dry whole milks a low temperature of storage is not an important factor. The work of Dahle and Palmer (1) is the only one reported wherein the effect of temperatures upon the rate of deterioration in dry whole milks has been noted. Their results are qualitative in nature only. They state that "the temperature at which the powders are stored proved to be an important factor. Not a great deal of difference was observed between powders stored at 4° and 20°C., but a great difference was observed when powders were stored at 37°C."

EXPERIMENTAL

A commercial sample of dry whole-milk powder was divided into two parts. One part of 1.54 per cent moisture content was divided into six samples, and each was stored at various temperatures ranging from 3° to 30°C. The other half of the commercial sample was divided into small lots and adjusted to higher moisture contents. The final moisture contents ranged from 2.60 to 3.40 per cent. Six samples from this lot were stored at the same temperatures as the samples of lower moisture content. The susceptibility to oxidation or the induction period of each

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sample was determined from time to time over a period of six months. (Lower induction period indicates increased susceptibility to oxidation.) The results of these experiments are shown in figures 1 and 2.

The first noticeable difference in the results with low and relatively high moisture content powders is that in the samples of low moisture content the increased susceptibility is greatest in the first sixty days of storage, while in those of higher moisture content the rate of deterioration is retarded during the first sixty days, but thereafter it is more rapid for the higher temperatures. The rate is more regular at lower temperatures. There is an indication that low temperatures retard the reaction fundamentally concerned in the oxidation when free moisture is present. Though the results shown in figure 2 are too inconsistent within themselves to warrant specific conclusions or calculations as to the effect of different moisture contents, a general comparison of the results with high and low moisture contents confirms our former observations that free moisture retards the rate of de-

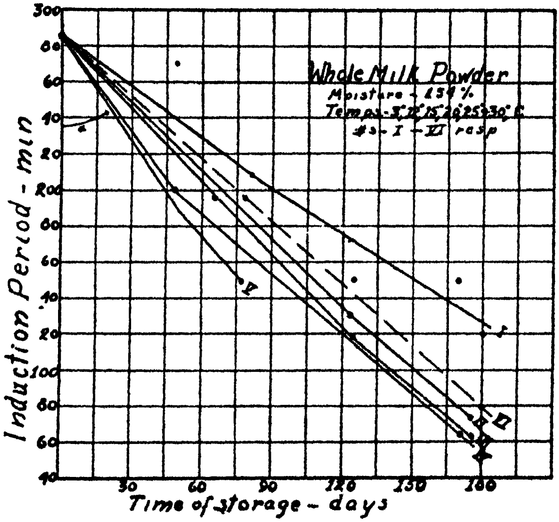

FIG. 1. SHOWING THE INCREASE IN SUSCEPTIBILITY OF THE FAT IN MILK POWDER OF LOW MOISTURE CONTENT TO OXIDATION DURING STORAGE AT VARIOUS TEMPERATURES

terioration (2). This point is discussed later. In each case, for some unknown reason, results with sample 6 differ from those of the other samples, and this sample is therefore not considered representative of each lot.

Though the moisture content of the samples used in obtaining the values shown in figure 1 are not considered optimum for the best keeping quality of this milk, a further study of these values gives an idea of the temperature effects upon the rate of the oxidation reaction where it is not complicated by the presence of any appreciable amount of free moisture.

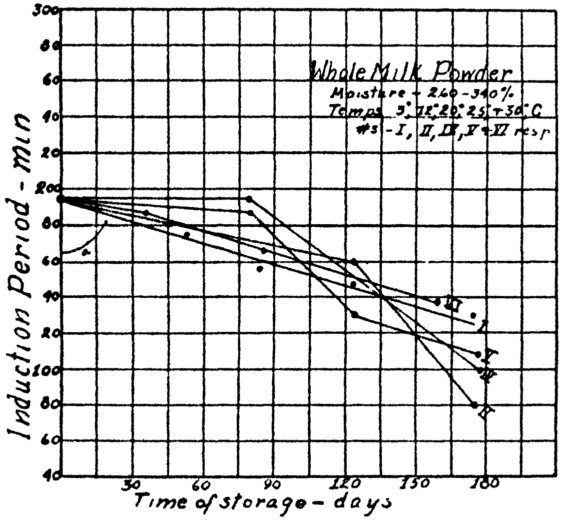

FIG. 2. SHOWING THE INCREASE IN SUSCEPTIBILITY OF THE FAT IN MILK POWDER OF RELATIVELY HIGH MOISTURE CONTENT TO OXIDATION DURING STORAGE AT VARIOUS TEMPERATURES

The rates of increased susceptibility to oxidation (lowered induction periods) shown in figure 1 are measured by the angles represented by α, which the line for each sample forms with the ordinate. The greater the angle the less rapid is the rate of increased susceptibilities, and vice versa. Thus for sample 1 stored at 3°C. the rate is considerably less (greater angle α) than it is for sample 5 at 25°C. (smaller angle α). Numerical values for the rates of susceptibility changes are therefore obtained by ascertaining the tangents of each angle. When these values are plotted against the temperatures of storage the relative value of each temperature over the higher temperature is noted.

Figure 3 indicates a regular increased keeping quality for each 5°C. decrease in the storage temperature between 25° and 10°C.

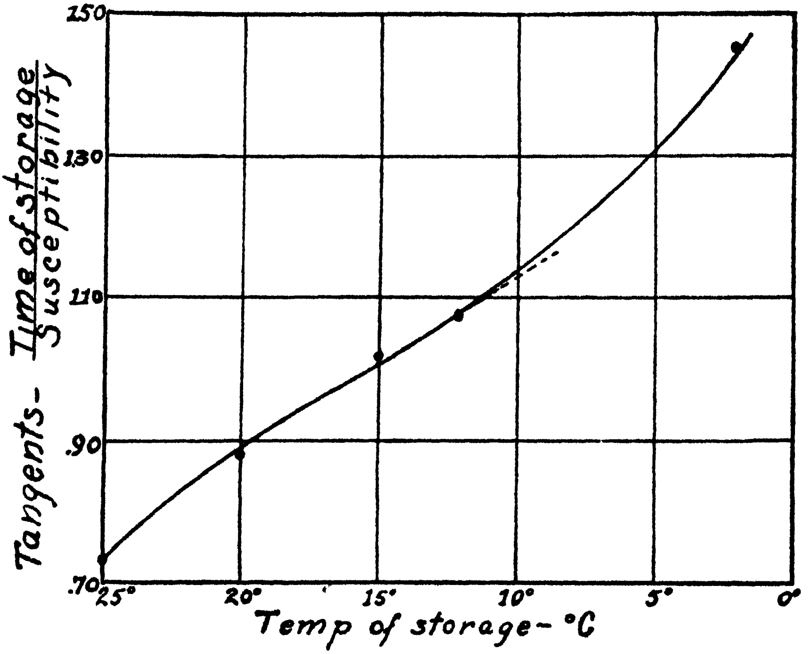

FIG. 3. SHOWING THE CHANGES IN TIME-SUSCEPTIBILITY RELATIONSHIP WITH TEMPERATURE OF STORAGE

Below 10°C. a marked increase in keeping quality is shown for each 5°C. decrease in storage temperature.

It may be stated that the dry whole milk of exceedingly low moisture content stored at 3°C. increased in susceptibility at approximately one-half the rate of the sample stored at 25°C. (tangents 1.45 and 0.72 respectively). This is also graphically shown in figure 1. A drop in the induction periods to any chosen value requires approximately twice as long a storage time at 3°C. as it does at 25°C.

With regard to dry whole milks of higher moisture content, another factor, namely, free moisture, is being dealt with. This factor affects the rate of the oxidation. As stated heretofore, the results shown in figure 2 are too inconsistent to warrant quantitative treatment. This figure, however, indicates that the rates are considerably less than those in figure 1, since the slopes of curves are greater with respect to the ordinate. Sample 1 in figure 2 shows a uniform rate of increased susceptibility. The tangent of the angle that this line forms with the ordinate is 4, while that of sample 1 in figure 1 is 1.4. This indicates that at 3°C. the rate of deterioration of a dry whole milk of exceedingly low moisture content is roughly 3 times as rapid as it is in a milk of higher vapor pressure where free moisture is present. The average rate of increased susceptibility for nos. 4 and 5 after eighty-five days storage is approximately equal to the rate of sample 1 (stored at 3°C.) and of figure 1, as expressed by the values of their slopes, 1.44 and 1.40 respectively. These figures indicate that moisture is a very critical factor in the study of keeping quality, especially in the region of 1.5 and 2.5 per cent.

These results confirm conclusions which the authors have reached in a previous work—that the optimum moisture content for spray powders of good solubility with respect to keeping quality is between 2 and 3 per cent. For powders with reduced solubility the optimum moisture content for increased keeping quality would be somewhat less, the vapor pressures being the same.

The subject of storage of products containing fats and oils, in vacuum or in inert gases, is one of considerable interest to the industry. Previous experiments with materials stored in carbon dioxide and in vacuum had given indefinite results. With a more accurate method available for measuring the changes that underlie deterioration and with a better knowledge of factors to be controlled, attempts again were made to determine quantitatively the rates of changes in susceptibility to oxidation during a storage in air, in partial vacuum, and in carbon dioxide.

Samples of the same dry whole milks of low and high moisture content were used in this experiment as were used in our previous experiments upon temperature of storage. All samples were stored at temperatures ranging from 21° to 23°C. Samples were opened from time to time and the susceptibility to oxidation (induction period) was determined. The time when the first off odor was perceptible was also noted and is designated with an × in figure 4. An increase in the intensity of off odor is designated by an increase in the size of the character.

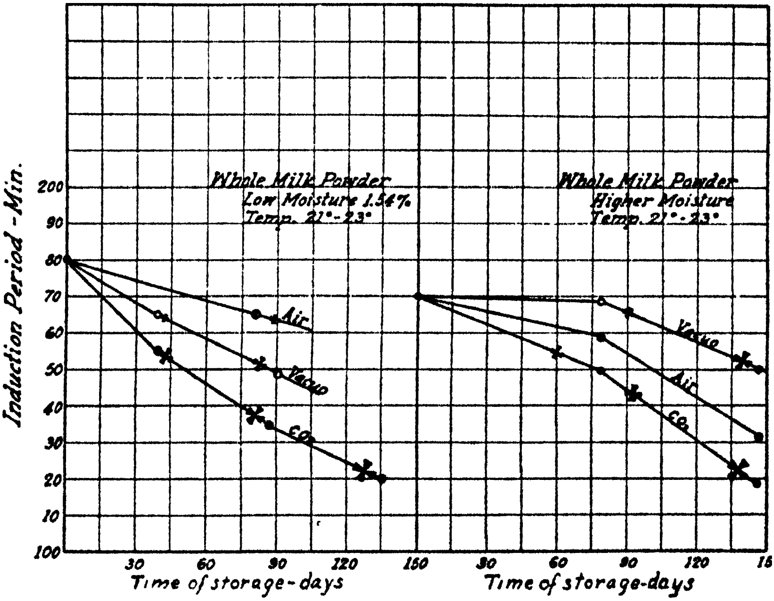

FIG. 4. SHOWING THE EFFECT OF VARIOUS ATMOSPHERES UPON THE SUSCEPTIBILITY OF THE FAT IN MILK POWDER TO OXIDATION DURING STORAGE

No great difference was noted between storage in partial vacuum and in air. Figure 4 indicates that for high and low moisture their relative values are reversed. In each case, however, storage in carbon dioxide proved the least efficient, the rate of susceptibility increase (lower induction period) being greater than for the other conditions shown. When judged by the olfactory sense dry whole milk stored in carbon dioxide always produced off odors and flavors before they were detectable in the samples stored in air or in partial vacuum.

These results are in accordance with what might be expected when the question is considered in the light of the effect of carbon dioxide upon soaps. It has been shown (3) that carbon dioxide can affect the splitting or hydrolysis of soaps (sodium salts of the fatty acids) and it seemed probable that the glycerides (glycerine + fatty acids) might also be affected in the same manner. This would produce free acids which are catalysts for the autoxidation of fats. These observations upon dry whole milks have been confirmed by results obtained upon pure milk fat.

DISCUSSION

The relative values for the susceptibility of fats to oxidation when stored at various temperatures, as shown in figure 1, were obtained upon dry whole milk which had not received clarifying treatment. Data previously presented indicated that this treatment of a milk materially improved the keeping quality of its dry whole milk. What effect this process may have upon the reaction involved is not known. The initial change that occurs in oxidation is undoubtedly modified greatly by the enzymes present, which are to a great extent removed in clarification. The reaction dealt with in the clarified product is therefore probably one of a different order and affected to a different degree by temperature changes. Further work will determine the variations of changes in the two products at different temperatures.

Results with dry whole milks of a low and relatively high moisture content indicate that free moisture has a decidedly retarding effect upon susceptibility to oxidation. For optimum keeping quality, therefore, a slight amount of free moisture is necessary. Since no two powders manufactured by different processes are alike with respect to their water adsorption capacities, each powder would have its own optimum.

For spray powders of good solubility the optimum moisture content seems to be between 2 and 3 per cent. The upper limit which can be used will be determined by several factors, namely, at what moisture content hydrolysis is promoted, and at what moisture contents solubility is affected by storage.

The data upon storage in various atmospheres are too meager to furnish any quantitative relationships. Repeated experiments with butteroil have confirmed the observations recorded. The results indicate that carbon dioxide can not be classed as an inert gas when its effect upon fats and oils are dealt with.

The results obtained with the samples stored in vacuum confirm previous results obtained in this laboratory. They indicate that keeping quality is largely dependent upon the inherent quality of the fat. The nature of the condition producing this inherent keeping quality has been shown to be due to some type of loosely bound oxygen which can not be removed by the use of vacuum. The amount of oxygen present in this form is usually sufficient to cause perceptible odors and flavors when it oxidizes the fat. Experiments with steam treated milk fat, reported in a former publication (4), tend to show this fact. Later experiments of more direct bearing upon this question are being published elsewhere (5).

Clarification seems to remove the catalysts necessary to the formation of these compounds and therefore improves the inherent quality of the fat. The effect of vacuum storage of a clarified product would probably therefore not be comparable to the results reported here.

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A METHOD OF INTERPRETING THE SCORES OF JUDGES OF DAIRY PRODUCTS*

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When competent judges are scoring cheese which have been made for experimental purposes, they are usually urged to place their scores upon each cheese without reference to the scores which other judges place upon the same cheese. Under such circumstances the judges will rarely score the cheese on exactly the same scale. The differences which result may be even greater than those which will be considered in this discussion. The determination of the significance of such scores is difficult and the problem is not limited to the scoring of cheese. It is encountered whenever an attempt is made to interpret the opinions of judges of milk, butter or ice cream when their judgments are recorded as numerical values upon more or less variable scales.

That the scores of the same cheese by different judges do not show exact agreement is illustrated in table 1, in which is presented a summary of the scores which three qualified judges placed upon a lot of 117 different cheese. The judges scored the cheese independently except when occasional comments were made upon exceptional samples.

Table 1 indicates rather strikingly that these judges agree upon neither the average score of the cheese nor upon the amount of variation in the scores of the series.

When the individual scores of each judge were examined, it was found that they were not using the same scale of points in judging the cheese. This is indicated in table 2 where the extreme variations of the judges' scores are shown.

From the combined evidence of tables 1 and 2, it is apparent that the mental standards of the judges in scoring cheese do not coincide. It does not follow that these judges are unable to

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select the best cheese and the poorest cheese and to place in their proper relative positions the cheese of all the intermediate grades. It is apparent, however, that the quality of a cheese scored 86 by A is not the equivalent in quality to the cheese which either B or C would designate by the same score. Judge A uses 17.75 points of score to indicate the same range of quality which B scores within a range of 12.50 points and C within 12.75 points. A point of score on the scale of points used by each of these judges should have the same significance to be comparable.

TABLE 1

The mean scores of 117 cheese estimated by three judges

JUDGE	MEAN SCORE	STANDARD DEVIATION	COEFFICIENT OF VARIATION
A	89.26 ± 0.19	3.03 ± 0.13	3.39
B	93.25 ± 0.18	2.91 ± 0.13	3.12
C	89.95 ± 0.16	2.58 ± 0.11	2.87

TABLE 2

The range of scores used by the three judges

JUDGE	SCORE OF THE BEST CHEESE	SCORE OF THE POOREST CHEESE	DIFFERENCE IN SCORE BETWEEN THE BEST AND POOREST
A	95.75	78.00	17.75
B	97.75	85.25	12.50
C	95.25	82.50	12.75

The fact that these judges examined the same lot of cheese must be emphasized. Since the range of score of each judge indicates the difference in quality between the best and the worst cheese, it seems logical to assume that the range of score of each judge should be numerically equivalent. These ranges of scores can be made numerically equivalent by assuming that the range in quality exhibited by the cheese of the series is equal to 100 points on a new scale in which the poorest cheese scores 0 and the best cheese scores 100. On the basis of this assumption it can be stated that A's range of score, which is 17.75 points, will equal 100 points on the new scale. B's range of score and C's range of score of 12.50 points and 12.75 points respectively, will also equal 100. The score of any cheese of any judge can now be transposed to this common scale of points by a simple calculation which as a formula can be stated as:

$$x = \frac{100\,(s - m)}{r}$$

In this formula x equals the score of the cheese on the scale of 100; r equals the maximum actual score of the judge minus his minimum actual score; s equals the actual score of the cheese; and m equals the minimum actual score of the judge.

TABLE 3

The actual and ratio scores of 10 of the 117 cheese examined by the three judges

CHEESE NUMBER	JUDGE A		JUDGE B		JUDGE C	
	Actual score	Ratio score	Actual score	Ratio score	Actual score	Ratio score
1	91.50	76.0	92.50	58.0	89.25	52.9
2	88.00	56.3	94.25	72.0	91.50	70.6
3	88.50	59.1	92.50	58.0	92.00	74.5
4	90.50	70.4	96.00	86.0	91.00	66.6
5	88.50	59.1	95.25	80.0	89.50	54.9
6	95.75	100.0	97.25	96.0	95.25	100.0
7	90.75	71.8	94.50	74.0	91.00	66.6
8	94.25	91.5	95.75	84.0	95.00	98.0
9	95.50	98.6	96.75	92.0	95.00	98.0
10	93.25	85.9	96.25	88.0	93.50	86.3
Average......	91.65	76.9	95.10	78.8	92.30	76.8

This "ratio score," as it might be called, indicates the quality of a cheese relative to the quality of all the other cheese in the lot examined.

To illustrate the application of this formula, the actual scores of judges A, B, and C on ten cheese selected at random from the 117 examined are presented in table 3 with the equivalent ratio scores calculated from the value of r and m given in table 2. Judge A, for example, gives cheese no. 1 in table 3 an actual score of 91.50. His maximum actual score, 95.75, less his minimum actual score, 78.00, gives a value for r of 17.75. Substituting in the formula:

$$x = \frac{100\ (91.50 - 78.00)}{17.75} = 76.00$$

This ratio score method of interpreting the significance of the scores of the cheese judges is not advanced with the idea that it is a perfect solution of the problem but rather as a tentative approach toward a common method of showing the results of experimental work where the quality of the product is involved. The method may be criticized adversely because it depends on the extreme range of the actual scores. Judges may allow their liking for a good product to influence unduly their highest score and may also cut too severely the score of the poorest sample. On the other hand this treatment of the judges' scores gives a series of numerical values which indicate the quality of the cheese examined on a scale of points common to all the judges. These values may be more correctly used than the judges' actual scores in determining the mean, standard deviation or the probable error of the series.

A PHOTOGRAPHIC METHOD FOR OBTAINING ACCURATE MEASUREMENTS OF ANIMALS*

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In connection with certain feeding experiments under the direction of Prof. H. B. Ellenberger,[1] it seemed desirable to obtain photographic records of the size and condition of the animals at frequent intervals. In pictures of this sort which have been made hitherto, the measurements usually have been indicated by a background, placed behind the animal, and ruled off in squares, or by a screen of intersecting wires placed in front of the animal. Both of these methods are open to criticism since they do not give a true measurement. The ruled background makes the animal appear larger than it actually is, while the screen in front reduces the apparent size.

This condition is shown diagrammatically in figure 1. The line *CC* represents most nearly the true measurement of the animal, the shorter line *AB* the size indicated by the screen, placed in front, and the longer line *A'B'* the size as shown on the background.

In order to avoid the error involved by the use of either of the two methods just described the writer worked out a method which records the actual size of the animal. Bars, scaled off at the desired intervals, were arranged to make a frame entirely surrounding the subject so that the animal could be placed in such a manner that the plane of the center of its body corresponded with that of the frame which gave the scale of measurement. An ordinary background was used and the frame was so made that it could be moved forward or backward and set at any desired distance away from the background. The accompanying pictures show the details of the construction of this apparatus.

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[1] Head, Department of Animal and Dairy Husbandry, University of Vermont and Vermont Experiment Station.

Figure 2 shows the frame surrounding the animal. The section of the frame resting on the platform upon which the animal stands is also shown in figure 5. The white marks on this section of the frame in this picture indicate the scale of measurement corresponding to that on the sides and top of the frame as shown in figures 2 and 4. Figure 3 shows the relative position of the frame and the animal. The bottom supports of the frame slide backward or forward in grooves and the position of the frame may be readily changed and fastened securely by means of ordinary clamps.

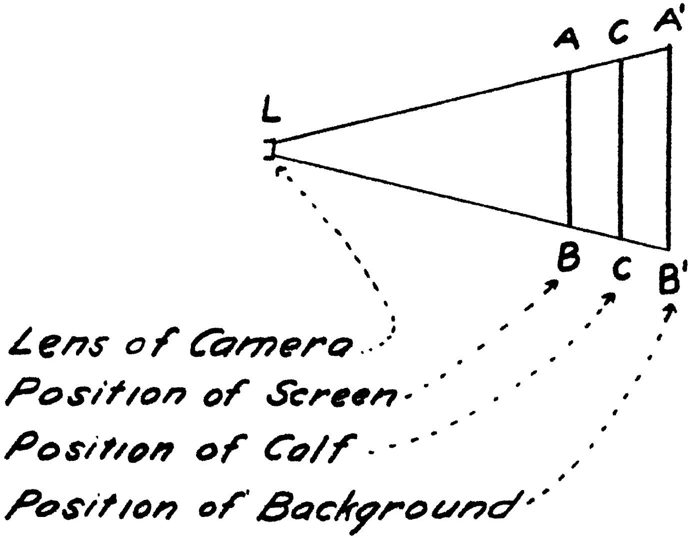

FIG. 1. THE OBJECT TO BE MEASURED MUST BE IN THE SAME PLANE AS THE SCALE OF MEASUREMENT

In order to have the camera at the desired place each time, an adjustable camera stand was built so that the camera could be set at a known height above the platform. A track was built in front of the platform running at exactly right angles to it. The platform and track were leveled up so that the camera would be perfectly square with the background in whatever position the camera was placed. The slide on the camera stand, and also the track were marked with a scale so that any position of the camera might be recorded and duplicated if desired.

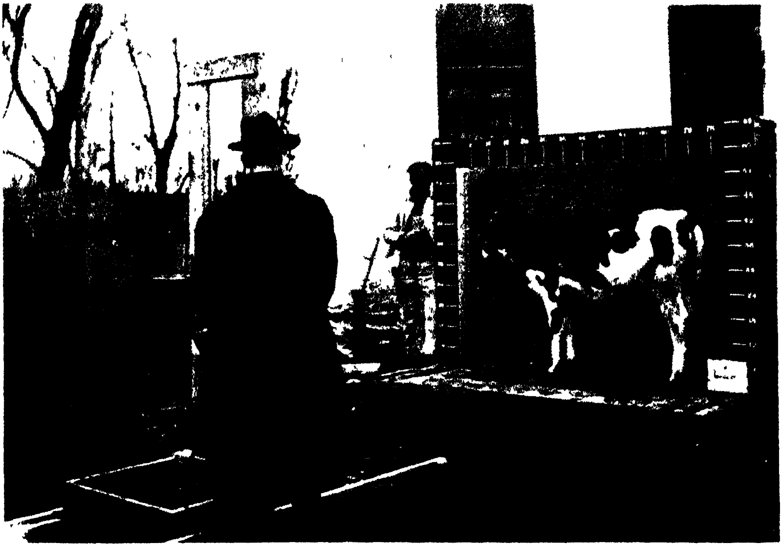

FIG. 2. THE ARRANGEMENT OF THE CAMERA, FRAME AND BACKGROUND

FIG. 3. THE POSITION OF THE ANIMAL IN RELATION TO THE FRAME

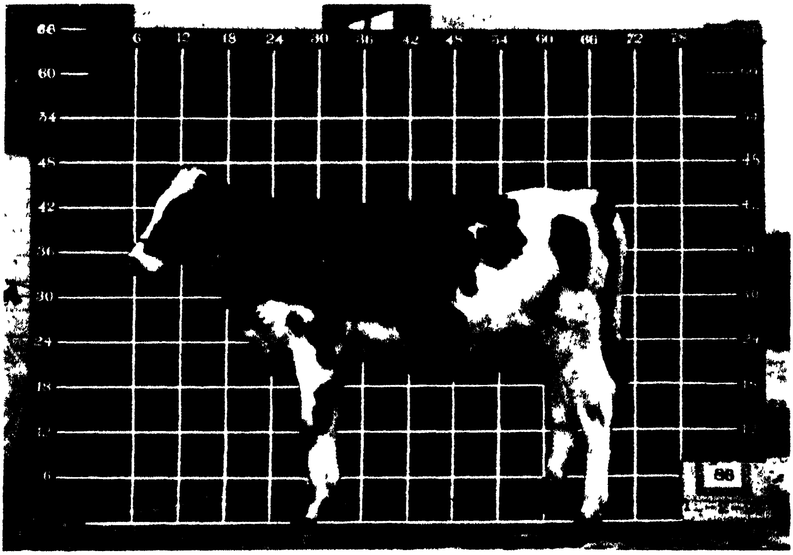

Fig 4 Measurements by the Improved Method

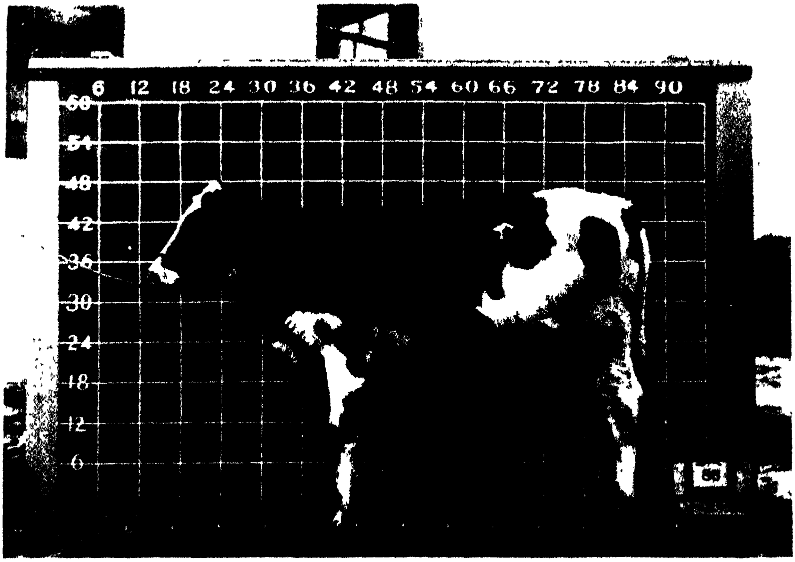

Fig 5. Measurements by the Ruled Background Method

After the negative is made the squares are ruled off with a ruling pen, using waterproof black India ink, and stopping when the line strikes any portion of the animal's body. Thus when the prints are made the lines show up white. In the case in question, as the calves were black and white, a gray background was used which would contrast with both the black and the white. Of course if it is desired, the lines can be ruled on the finished print.

The difference in the size of a six months' old calf photographied against a ruled background and by the improved method is shown in figures 4 and 5. Figure 4 illustrates the improved method and figure 5 the ruled background. The camera was exactly the same distance from the calf in each case but the size of the calf as indicated by the scale is quite different.

Although this method is not quite as simple as those that have been previously used, yet it would seem that the increased accuracy obtained is worth much more than the extra work involved.

STUDIES ON YEASTS IN DAIRY PRODUCTS

II. GENERAL GROUPING OF THE MORE NUMEROUS TYPES

W. A. CORDES[1] AND B. W. HAMMER

One of the objects of the studies that have been carried out by the dairy section of the Iowa Agricultural Experiment Station on the yeasts found in dairy products has been the eventual development of a system of classificaton that will make possible the easy and accurate identification of yeast cultures isolated. Because of the wide range of forms found among the yeasts such a classification necessitates a separation of the organisms into groups with those in each group having relationships, more or less close, that naturally throw them together. A grouping that has been in use for several years is herein presented with the idea that it may serve as a starting point in the development of a scheme of classification for the yeasts found in dairy products; a rearrangement and further division of the groups is to be expected but can only be made as a result of a detailed study of the organisms belonging to each.

The organisms in certain of the groups have been studied in considerable detail while those in other groups have not. The results obtained indicate that the grouping adopted, although incomplete, is of help in a consideration of the yeasts isolated from milk and cream and their derivatives.

Grouping of yeasts found in dairy products

A. Group I. Yeasts producing conspicuously colored colonies.
    a. Producing pink colonies.
        Includes *Torula glutinis* and also other forms.
    2a. Not producing pink colonies.
        b. Having yellow colonies due to associative action with *Aspergillus niger*.
            Various species of yeasts are here involved.
2A. Yeasts not producing conspicuously colored colonies.
    B. Group II. Yeasts producing dull, spreading, irregular-edged colonies on whey agar.

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- a. Growth very thin on whey agar; action on sweet milk inconspicuous.
  Includes *Mycoderma monosa*.
- 2a. Growth on whey agar flat but not thin as under "a"; rapid digestion of sweet milk.
  Includes a type commonly found but not yet described.

2B. Yeasts not producing dull, spreading, irregular-edged colonies on whey agar.

- C. Group III. Yeasts showing white, smooth-edged colonies and pronounced gas formation in milk.
  - a. Optimum near 37°C.; cells oval. *Torula cremoris*.
  - 2a. Growth slow if at all at 37°C., but good at 30°C.; cells spherical. *Torula sphaerica*.
- 2C. Group IV. Yeasts showing white, smoothed-edged colonies but *no* gas formation in milk—common white yeasts.
  - a. Gas produced is saturated sucrose bouillon.
    Includes *Torula lactis-condensi*, and probably other forms.
  - 2a. No gas in saturated sucrose bouillon.
    Includes a great variety of species many of which produce no change in sweet milk.

Groups I and II are separated from the other groups on the basis of the appearance of the colonies; this is done primarily for the reason that the first information in regard to a culture isolated relates to its colony appearance and accordingly any division that can be made on such a basis is very desirable. The division of the organisms not included in groups I and II cannot be made on the colony basis because there is nothing characteristic, but the organisms belonging to group III can easily be separated from those of group IV by inoculating into litmus milk and examining for gas formation; after some little experience the organisms of group III can be recognized by the odor produced in pure cultures in agar, milk, or other materials. Group IV is really a catchall group which further studies are certain to divide up; the organisms in this group apparently present the greatest difficulties from the standpoint of an adequate classification.

The groups established in the scheme are believed to be arranged in such a way that the one to which a given organism belongs can be determined in the easiest possible manner. The group of colored colony yeasts is put first because one of the most striking and easily noticed characters of a colony is its color; in young colonies the color production may not be as pronounced as in older ones, but any grouping involving cultural characters must necessarily be dependent on cultures that had been allowed a reasonable time to develop. In subsurface colonies the color of the pink yeasts is often not as pronounced as in surface colonies but ordinarily it is intense enough to be easily recognized in plates that are several days old. The dull, spreading, irregular-edged colony distinguishing group II is another striking character that is quite easily recognized and readily distinguished from the smooth-edged, shiny colonies found with the remaining groups. Groups III and IV cannot be separated on the colony basis but group III is characterized by lactose fermentation and this is easily determined by observing the changes occurring in inoculated milk. The odor produced during the lactose fermentation is quite characteristic so that the organisms belonging to group III can often be selected on this basis also.

Group IV, the common white yeasts, is the group which experience suggests will be certain to require further division. There are wide variations in the yeasts here included but the work so far done does not suggest any very logical basis for division. Distinct species can easily be separated but any relationship between these is not as yet clearly evident. The formation of gas from strong sucrose solutions, e.g., saturated sucrose bouillon, is a suggested basis for the first division, mainly because the yeasts effecting this change are so important in the sweetened condensed milk industry; however, there seem to be organisms that can ferment saturated sucrose bouillon that cannot ferment sweetened condensed milk.

The grouping given does not include all the yeasts that can be isolated from dairy products, but does include those that have been isolated often enough to be considered of significance. For example, a yeast producing a brown color was isolated from cream and there is no provision for this in the scheme of grouping, although a place could easily be provided. This yeast, however, has never been found again and while it is of interest because of the rarity of yeasts producing a brown color, a study of only one culture cannot give an adequate idea of it so that its description had best be left until other cultures are found or some special importance seems attributable to it. Descriptions of organisms based on only one culture cannot take into account the variations occurring in the species and are thus likely to lead to needless confusion in the literature.

THE USE OF VARIOUS FORMS OF OXYGEN IN THE TREATMENT OF ABNORMAL FERMENTATION IN SWISS CHEESE*

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One of the most common difficulties encountered in the manufacture of Swiss cheese is the development of the so-called nissler fermentation. A nissler fermentation is one in which the gas-producing organisms are present in such numbers or in such a state that the ordinary lacto bacilli in the whey rennet or "Lab," or the bulgaricus starter fail to hold the gas-producing organisms in check, and as a result the cheese does not have a firm, solid curd but is filled with numerous small holes sometimes one-sixteenth to one-eighth of an inch in diameter. Such a cheese is spoken of as a nissler, or a cheese with a thousand eyes. When a very vigorous and rapid fermentation takes place while the cheese is on the press so that the cheese huffs and may spread over the side of the press hoop, it is spoken of as a pressler cheese. Either of these fermentations may result from overripe or dirty milk, from contamination through unclean utensils, or even from the use of overripe or gassy "Lab" or "sour." The prevalence of these abnormal fermentations follows the seasonal temperatures closely. In general, the highest percentage occurs during the summer months; the lowest in the spring, fall, and winter months.

At Grove City, Pa., where milk is delivered once a day, resulting formerly in a high percentage of nissler cheese during the summer months, it has been found that during this period the number of such cheeses could be reduced to a comparatively low percentage by chilling the milk to 50°F. as soon as it reaches the creamery. In the case of milk delivered once a day, the nissler fermentation often manifests itself while the cheese is

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on the press and is ordinarily determined by sounding with a large spoon. In this case no huffing is in evidence. If a cheese sounds more or less over the entire surface instead of in one or two spots, it will very likely prove to be a nissler. Occasionally a cheese will sound over the entire surface and still not show any indications of gas except within an inch or so of the edge. Such a condition may be brought about as a result of too rapid cooling or of too frequent turning while on the press. When a cheese sounds in only one or two spots it is probably an indication of whey pockets which, as the name suggests, are merely isolated areas where whey collects and is not properly expelled.

Another means of determining whether or not a cheese is a nissler is by examining it with a small Roquefort trier at the time it is removed from the brine tank. If no holes are present in cheese made from milk delivered once a day, it probably is not a nissler, although this fermentation may occasionally develop later in the curing process.

In case of abnormal fermentations in cheese made from milk delivered twice a day, the cheese seldom sounds while on the press; and very careful examination is often required later to detect the minute holes which, when present, are a fairly certain indication of a nissler condition. Some manufacturers claim that these pin holes do not develop for several days after the cheese has been removed from the brine tank.

Pasteurization has been used to a limited extent in an effort to control these types of fermentation. A patented process, however, prevents the general use of the holding system of pasteurization of milk for the manufacture of Swiss cheese. Results obtained by the holding system of pasteurization have not always proved satisfactory as the cheese manufactured after the milk has been pasteurized often cracks or checks internally, and at times an abnormal fermentation may even develop causing it to be no better than a No. 2 grade. In general, pasteurization of milk for making this kind of cheese tends to delay the eye formation until the curd has lost some of its normal elasticity; and as a result, the cheese either checks or has a tendency toward becoming glaesler. A glaesler cheese is one that usually cracks or splits crosswise to the flat surface, or one near the surface of which small checks form which frequently are only an inch or so in length. Unless the cheese is made on an extensive scale the cost of equipment and the extra labor required makes the pasteurization of milk for Swiss cheese impractical for the average factory.

In factories receiving milk once a day it appears that it is the anaerobic rather than the coli-aerogenes types of organisms which are primarily responsible for these abnormal fermentations.

At present the most practical means of controlling these abnormal fermentations is by the production of clean, quickly cooled milk and the use of an active, pure bulgaricus culture. Even when these conditions prevail, cheeses of either the nissler or pressler type sometimes result.

Since bacteriological studies have often indicated the presence of anaerobic spore-forming bacteria in the milk and cheese, the use of some form of oxygen as the means of treating milk suggests itself. By adding gas-producing anaerobic cultures to milk, studies have been made on the effect of the use of ozone, oxygen and aeration in checking subsequent gassy fermentations in Swiss cheese.

THE USE OF OZONE IN THE TREATMENT OF MILK

Attention was first directed toward the possibility of utilizing gas in the treatment of abnormal fermentation occurring in milk as a result of an article on this subject by Emil Weiner and W. Freund (Vergl. W. Freund, Chem. Ztg., 1911, S. 905; and Weiner klin. Wochenschr., 1910, Nr. 26). A summary of Weiner's results is as follows: Very good results were obtained in the sterilization of milk by first atomizing the milk and then blowing through it a very strong concentration of ozonized air (0.03 to 0.4 gram per cubic meter) and following this by reatomizing the milk and blowing it thoroughly with sterile air so as to drive off the surplus ozone. In this way it is claimed that the treated milk was absolutely freed from all pathogenic bacteria. It appears that the acid bacteria of milk were not much affected but that the ozone was extremely active toward foreign organisms. Further claims are made that when milk is treated in this way, the taste and odor of the milk are not affected, nor are the vitamins or proteins. Doctor Freund also used ozone in his attempts to sterilize milk by running ozonized air through milk for a period of ten minutes. This treatment rendered the taste and odor of the milk so offensive that it could not be used for practical purposes. In order to use ozone it is necessary to atomize the milk so as to expose a maximum surface. It is also essential to dispose immediately of the surplus ozone by reatomizing the milk and blowing it with sterilized air.

The practicability of atomizing milk in order that it might be more effectively treated with ozone and further treated with sterile air did not seem to offer any possibilities, at least so far as the manufacture of Swiss cheese is concerned. There seemed a possibility that the injurious effects of the ozone might be dissipated during the process of manufacture and curing of cheese.

METHOD OF TREATMENT

All milk used in these experiments was first mixed in a large holding tank, weighed, and equal quantities run into small experimental kettles, each holding approximately 700 pounds. The milk was thoroughly mixed with the exception of a small portion used for standardizing purposes. This milk was put into a kettle and heated to the proper temperature, separated, and then equal portions added to the experimental kettles. Each kettle received similar quantities of the Bacillus bulgaricus, the eye culture, and rennet in suitable proportions. In addition to the usual Swiss starter a gas-producing spore-forming anaerobe which was originally isolated from a Swiss cheese was also added. The purpose of this organism was to develop a gassy fermentation in the cheese. Only one kettle was treated with oxygen, the other served as a check. Each pair of cheeses was manufactured as nearly alike as possible. The quantity of milk used each day varied somewhat, but usually 450 to 650 pounds of milk were used for each cheese.

The ozone was prepared by running air first through sulphuric acid and then through an ozone machine in which currents of air passed between two highly charged electric plates. The ozone thus generated was conveyed from the machine to the milk by means of a glass tube with connections sealed with paraffin. The ozone was not as a rule run through the milk vigorously but at a fairly uniform rate of possibly 15 to 20 cubic feet per half hour the milk being stirred meanwhile by means of an agitator. At the conclusion of the ozone treatment, air was blown through the milk rather vigorously for five to ten minutes. It was possible to record only the time at which the ozone was run in, or the number of cubic feet of the gas used, since the quantity of ozone actually absorbed by the milk could not be measured. As indicated in table 1 ozone was run into milk for periods varying from fourteen to forty-five minutes. Since the presence of ozone gas coming from the milk was easily discernible by its characteristic odor, there could be but little question as to when the gas was generated. With the exception of a few cases, the same quantity of milk was used in each kettle. In these cases the difference in the weight of the milk was only about 20 pounds so that this variation would not seem sufficiently great to influence appreciably the final results.

The ozone was run into the milk at temperatures varying from 3° to 33°C.; in a few cases no record was made of the temperature. When this was done the milk was not warmed and seldom ran above 12°C. In all cases the temperature of the check kettle was held as nearly like that of the treated kettle as possible. The anaerobic culture was added to the milk just before the introduction of the ozone gas, the bulgaricus and eye cultures just before the addition of the rennet.

DISCUSSION OF RESULTS

Table 1 gives the results obtained by adding an anaerobic culture to milk and treating it with ozone. About 44.44 per cent of the cheeses showed no gas, 38.88 per cent showed improvement while 16.66 per cent showed no improvement as compared to the check cheeses. Seventy-five per cent of the ozonated cheeses had an off flavor, while 25 per cent showed no off flavor.

In many cases the cheeses were huffed so much that the edges

TABLE 1

Effect of ozone on the flavor and gassy fermentations of Swiss cheese

NUMBER	QUANTITY OF MILK	QUANTITY OF GAS CULTURE	LENGTH OF TIME OZONE RUN	TEMPERATURE OF MILK WHEN TREATED	ACIDITY BULGARICUS CULTURE	GRADE OF CHEESE*	EFFECT OF OZONE†	PRESENCE OF OFF FLAVOR‡	REMARKS
	pounds	*cc.*	*minutes*	°*C.*	*per cent*				
1	600	1,350	30	Below 12	1.70	No. 1 g	+		Slight off flavor
1-1§	600	1,350	None		1.70	No. 1 f		Yes	Nissler outer edge
2	600	*	45	Below 12	1.52	No. 1 p	−	Yes	Slight off flavor
2-1§	600		None	Below 12	1.52	No. 2			
3	525	1,000	30	Below 12	0.76	No. 2	0	—	Pressler cut morning after making
3-1§	525	1,000	None	Below 12	Whey	No. 2			Pressler cut morning after making
4	650	1,000	14	30		No. 2	0		Nissler edge. Possibly too little ozone
4-1§	650	1,000	None	30		No. 2		—	Nissler edge
5	575	800	30	30	1.53	F. f	+		No off flavor
5-1§	575	800	None	30	1.53	No. 1 p		No	Nissler at edge
6	540	1,300	45	30	1.52	F. f	−		Not nissler. No off flavor
6-1§	540	1,300	None	30	1.52	No. 1 g		No	Not nissler. No off flavor
7	510	800	45	15	1.30	No. 1 f	+		Flavor hopelessly off
7-1§	510	800	None			No. 1 g		Yes	Trace of nissler at edge
8	540	1,250	30	Below 12	1.60	F. p	+		Flat poor flavor not nissler
8-1§	540	1,250	None		1.60	No. 2		No	Nissler edge

9	530	925	45	15	1.30	No. 1 p	X		Very much off flavor. Little nissler at ends
9-1§	530	925	None	15	1.30	No. 2		Yes	Nissler 2 inches at ends
10	550	950	45	15	1.40	No. 2	X		Trace of nissler, cut morning after making
10-1§	550	950	None	15	1.40	No. 2		–	Slightly gassy throughout
11	650	1,225	30	25	1.36	No. 1 f	+		Ozone flavor plainly noticeable. Not nissler
11-1§	650	1,225	30	25	1.36	No. 2		Yes	Nissler 2 or 3 inches at edge
12	600	1,000	30	30	1.65	No. 1 g	X	No	Trace of nissler. Very little if any off flavor
12-1§	600	1,000	30	30	1.65	No. 1 f			Somewhat nissler at edge
13	525	800	30	25	1.32	No. 1 f	+		Distinct ozone flavor. Not nissler
13-1§	525	800	30	25	1.32	No. 1 f		Yes	Trace of nissler
14	530	850	45	Below 12	1.30	No. 2	X	–	Somewhat nissler. Cut morning after making
14-1§	530	850	45	Below 12	1.30	No. 2			Less nissler. Cut morning after making
15	625	950	23	33	1.72	No. 1 p	X		Somewhat nissler. Very much off flavor
15-1§	625	950	23	33	1.72	No. 2		Yes	Pressler. Cut morning after making
16	640	1,000	30	30	1.70	No. 1 f	+		Not nissler. Distinct off flavor
16-1§	657	1,000	30	30	1.70	No. 2		Yes	Nissler 1 or 2 inches at edge

TABLE 1—*Continued*

NUMBER	QUANTITY OF MILK	QUANTITY OF GAS CULTURE	LENGTH OF TIME OZONE RUN	TEMPERATURE OF MILK WHEN TREATED	ACIDITY BULGARICUS CULTURE	GRADE OF CHEESE*	EFFECT OF OZONE†	PRESENCE OF OFF FLAVOR‡	REMARKS
	pounds	*cc.*	*minutes*	*cc.*	*per cent*				
17	600	1,000	30	31	1.50	No. 2	0	–	Both cheese nissler. Bulgaricus added before ozone
17-1§	600	1,000	30	31	1.50	No. 2			
18	640	1,000	30	30	1.40	No. 1 f	+		Not nissler. Distinct off flavor
18-1§	640	1,000	30	30	1.40	No. 2		Yes	Somewhat nissler and very much over set
19	600	1,100	30	30	1.75	No. 2	X		Trace of nissler. Has ozone flavor
19-1§	600	1,100	30	30	1.75	No. 2		Yes	Pressler and nissler
20	676	1,000	30	30	2.01	No. 2	X		Trace of nissler. Distinct off flavor
20-1§	704	1,000	30	30	2.01	No. 2		Yes	More or less gassy throughout
21	580		30	30	1.48	No. 1 f	–		Distinct off flavor
21-1§	580		30	30	1.48	No. 1 g		Yes	

* F = fancy (Fancy and No. 1 further divided: g = good; f = fair; p = poor).

† + = completely checked; X = improvement; 0 = no improvement; – = no comparisons could be made.

‡ – = no comparisons could be made.

§ check cheese.

extended over the edge of the hoop and were so badly pressler that further experimentation could not be carried on, and they were accordingly cut for examination. In a few cases the cheeses showed nissler tendencies throughout, but more often the gas was only in evidence for a few inches at the edge, or at those points where the cheeses cooled the quickest. With large cheeses weighing over 100 pounds there is often a difference of from 2° to 3°C. between one point of the cheese and another. In a few days there was only a trace of gas in the interior, a condition which may occasionally happen whether or not gas cultures are added to the original milk. The cheese indicated by the positive sign in table 1 showed no gas either at the edge or at any point on the interior.

In cheese 3 a whey starter was used instead of the usual skim milk starter. In this particular case the acidity was somewhat higher than that usually employed with whey starters. A very high acidity in either whey or skim milk starter caused by too long an incubation period may weaken the organisms to such an extent that they fail to function normally.

In cheese 4 the ozone was run into the milk for fourteen minutes whereas in most cases it was run for about thirty minutes. This may have been an insufficient treatment of the milk.

In cheese 17 the bulgarian culture was added to the milk prior to the treatment with ozone instead of later, as was done in all other cases. It is possible that the ozone may have influenced the development of the bulgarian culture.

In those cases where an improvement was noted as a result of the ozone treatment, the check cheese showed gas to a greater distance from the surface, or to a greater degree.

There was a distinct off flavor in 75 per cent of the ozone-treated cheese. The flavor of the cheese was comparable to the characteristic odor of the gas. The intensity of the flavor in the ozonized milk cheese seemed to vary somewhat from day to day even when the milk was treated in the same manner. Generally, however, the defective flavor was so pronounced as to render the cheese unmarketable. Four of the ozonized milk cheeses showed no noticeable off flavor, but in most cases an unpleasant flavor,

TABLE 2

Effect of oxygen on the gassy fermentation in Swiss cheese

NUMBER	QUANTITY OF MILK	QUANTITY OF GAS CULTURE	LENGTH OF TIME OXYGEN RUN	QUANTITY OF OXYGEN	TEMPERATURE OF MILK WHEN TREATED	GRADE OF CHEESE*	EFFECT OF OXYGEN†	REMARKS
	pounds	*cc.*	*minutes*	*cubic feet*	*°C.*			
1	500	1,400	30		16	No. 2		Pressler
1-1†	500	1,400		None		No. 2	0	Pressler
2	419	850	20		16	F. p		Not nissler
2-1‡	419	850		None		F. f	+	Nissler at edge
3	460	1,050	20		15	No. 2		Not nissler
3-1‡	460	1,050		None		No. 2	+	Nissler at edge
4	460	1,025	20		9	No. 1 f		Not nissler
4-1‡	460	1,025		None		No. 1 f	+	Nissler at edge
5	360	725	15		9	No. 2		Pressler
5-1‡	360	725		None		No. 2	0	Pressler. No difference
6	369	1,000	20		8	F. f		Not nissler
6-1‡	369	1,000		None		No. 2	+	Pressler. Cut morning after making
7	369	950	15		8	No. 1 f		Nissler at edge
7-1‡	369	950		None		No. 2	X	Pressler
8	533	1,100	10		8	No. 1 f		Not nissler
8-1‡	510	1,100		None		No. 1 f	+	Trace of nissler
9	370	2,000	20		6	No. 1 f		Not nissler
9-1‡	370	2,000		None		No. 2	+	Pressler

10	370	1,000		10	6	No. 2		Nissler about 1 inch at edge
10-1‡	370	1,000		None		No. 2	X	Nissler throughout
11	370	1,000		20	7	No. 1 f		Not nissler
11-1‡	370	1,000		None		No. 1 g	+	Nissler at edge, 1 to 2 inches
12	370	1,000	–	10.5	7	No. 2		Pressler
12-1‡	370	1,000	–	10.5		No. 2	0	Pressler. No difference
13	370	1,000	–	11 2	8	No. 1 f	+	Not nissler
13-1‡	370	1,000		None		Cut		Pressler. Thrown out morning after making
14	370	1,000	–	11.2	8	No. 2	0	Nissler and pressler
14-1‡	370	1,000	–	None		No. 2		Nissler and pressler
15	370	1,000	–	17	8	F. p	+	Not nissler
15-1‡	370	1,000		None		Cut		Pressler. Cut morning after making
16	370	1,000	–	17	6	F. f	+	Not nissler
16-1‡	370	1,000		None		No. 1 p		Nissler at edge
17	370	1,000	–	17	6	F. p		Trace of nissler
17-1‡	370	1,000		None		No. 1 p	X	Nissler 2 or 3 inches from edge
18	370	1,000	–	18	10	F. p.	+	Not nissler
18-1‡	370	1,000		None		F. g		Nissler at edge
19	370	1,000	–	9.6	6	No. 2	0	Nissler at edge
19-1‡	370	1,000		None		No. 2		Nissler at edge
20	370	1,000	–	20.5	8	No. 2	X	Nissler at edge
20-1‡	370	1,000		None		No. 2		Nissler throughout

TABLE 2—*Continued*

NUMBER	QUANTITY OF MILK	QUANTITY OF GAS CULTURE	LENGTH OF TIME OXYGEN RUN	QUANTITY OF OXYGEN	TEMPERATURE OF MILK WHEN TREATED	GRADE OF CHEESE*	EFFECT OF OXYGEN†	REMARKS
	pounds	*cc.*	*minutes*	*cubic feet*	*°C.*			
21	370	1,000	–	20	13	No. 2	X	Nissler at edge
21-1‡	370	1,000		None		No. 2		Nissler throughout
22	370	1,000	–	23	16	F. f	+	Not nissler
22-1‡	370	1,000	–	None		No. 2		Genuine nissler with abortive eyes
23	370	1,000	–	17.5	16	F. p	+	Nissler
23-1‡	370	1,000	–	None		No. 2		Perfect nissler with abortive eyes
24	370	1,000	–	10.2	16	F. f	+	Not nissler
24-1‡	370	1,000		None		No. 2		One-half cheese nissler, pressler at edge and top and for 1 to 2 inches
25	370	1,000	–	5.5	17	F. f	+	Not nissler
25-1‡	370	1,000		None		No. 2		Nissler at edge
26	370	1,000	–	20.5	17	No. 2	X	Slightly nissler, center nissler
26-1‡	370	1,000		None		No. 2		Pressler and showed large holes
27	370	1,000	–	30	11	No. 1 g	+	Not nissler
27-1‡	370	1,000		None		No. 2		Genuine nissler with abortive eyes

* F = fancy (Fancy and No. 1 further divided; g = good; f = fair; p = poor.)

† + = completely checked; X = improvement; 0 = no improvement.

‡ check cheese.

which failed to disappear during the curing process, developed the day after making.

While the use of ozone might prove an economical means of treating milk containing anaerobes, the fact that the resulting cheese in most cases showed a distinct off flavor makes it appear doubtful whether such a gas could ever be used commercially for the treatment of milk used in the manufacture of Swiss cheese.

THE USE OF OXYGEN IN THE TREATMENT OF THE GASSY FERMENTATION IN SWISS CHEESE

In conducting these experiments oxygen was run into milk through a glass tube to which a perforated coil was attached, thus allowing a better distribution of the gas. The temperature of the milk varied from 6° to 17°C., the qualtity of gas used from 5.5 to 30 cubic feet per kettle, and the cheeses were made experimentally as with the ozone treatment. Table 2 gives the results obtained by adding a pure culture of a spore-forming anaerobe to milk and treating it with oxygen.

DISCUSSION OF RESULTS

Somewhat better results were obtained from the use of about 20 cubic feet of oxygen instead of 10 cubic feet. However, as in the case of the ozone, oxygen did not always check the gassy fermentation. Negative results were obtained when oxygen was added to the milk at both a high and a low temperature.

The effect of oxygen in the treatment of milk to which pure cultures of gas-producing spore-forming anaerobes were added is summarized as follows:

In about 59.25 per cent of the cheeses made the gas was completely checked by oxygen, in 22.22 per cent there was an improvement, and in 18.51 per cent no improvement was noted as compared to the check cheeses.

Nine pairs of experimental cheeses were made on a factory scale. One half of them received oxygen, while the remainder were used as controls. The milk was first mixed in a holding vat, divided, and then manufactured into cheeses as nearly alike as possible. Unfortunately from an experimental standpoint, only two cheeses showed nissler tendencies. With these two pairs of cheeses there was a striking contrast. In one case the oxygen-treated cheese was a good No. 1, in the other case a fancy, whereas the untreated cheeses were both No. 2. Neither of the No. 2 cheeses was, strictly speaking, pin-eyed nissler, but both were filled with numerous small eyes perhaps ⅛ to ¼ inch in diameter. These cheeses were made from 1550 to 1650 pounds of milk; the oxygen-treated cheese received 100 cubic feet of the gas in the original milk. In the other seven pairs of cheeses there was but little difference between the treated and untreated cheeses. The results here seem sufficiently favorable to warrant further experimentation to determine just how small a quantity of oxygen might be used to give effective results.

During the initial stage of eye development there was a marked contrast in eye formation between the nine pairs of treated and untreated cheeses, but upon final examination this earlier improvement was less marked except in the two cases above mentioned. These cheeses were all cut and sold locally, thus affording a good opportunity for careful examination.

One advantage in the use of oxygen is the fact that it has little or no injurious effect upon the flavor of the cheese under the laboratory conditions of the Bureau of Dairy Industry. With quantities of oxygen used it would cost about $1.00 to treat the milk for 150 to 160 pound cheeses. It is possible that in the experiments carried on in the laboratory when gas-producing cultures were added their number would be considerably in excess of what would normally be found in commercial milk. The gas cultures were grown in skim milk and thus particles of curd were introduced into the milk, a condition which does not afford so favorable an opportunity for oxygen treatment as in the case of commercial milk.

THE GERMICIDAL AND INHIBITORY ACTION OF OXYGEN

The action of oxygen on a spore-forming anaerobe used in these experiments indicates that the gas has a germicidal and inhibitory action on the organisms as indicated by dilution tests. Flasks containing 1000 cc. of milk were sterilized, cooled and inoculated with 2.5 per cent of the freshly grown gas-producing culture. Oxygen was run into half the flasks for periods of five, fifteen, and thirty minutes, while the remainder of the flasks served as checks. Dilution tests were then made in freshly sterilized milk tubes up to the sixth dilution from both the treated and untreated flasks. A period of one half hour was allowed to intervene after the oxygen treatment before transfers were made to the milk tubes, which were then placed in an incubator at 37°C. Four trials were made with dilution tests as indicated in table 3.

Dilution tests indicate some germicidal action in the tubes from flasks treated with oxygen for five and fifteen minutes and a rather marked action when treated for a period of thirty minutes. In one case the milk was sterile, and in another case growth occurred in the first dilution as a result of the oxygen treatment for thirty minutes.

TABLE 3

The germicidal and inhibitory action of oxygen on a spore-forming anaerobe as indicated by dilution test

PERIOD OF OXYGEN TREATMENT	TRIAL 1*						TRIAL 2						TRIAL 3						TRIAL 4					
5 minutes	+	+	+	−	−	−	+	+	−	−	−	−	+	+	−	−	−	−	+	+	+	+	+	−
No oxygen	+	+	+	+	−	−	+	+	−	−	−	−	+	+	−	−	−	−	+	+	+	+	+	+
15 minutes	+	+	+	−	−	−	+	+	−	−	−	−	+	+	−	−	−	−	+	+	+	+	+	−
No oxygen	+	+	+	+	−	−	+	+	−	−	−	−	+	+	+	+	+	−	+	+	+	+	+	+
30 minutes	−	−	−	−	−	−	+	+	+	−	−	−	+	−	−	−	−	−	+	+	−	−	−	−
No oxygen	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−

* + = growth; − no growth.

The original flasks treated with oxygen showed only a little, gas, and in the case of the thirty-minute treatment, practically none when incubated at 30°C. for twenty-four hours; whereas the corresponding check flasks showed the presence of gas to a marked degree. The oxygen-treated flasks gave a solid marblelike curd and very little if any gas was in evidence even after a period of several days. The action of oxygen on the spore-forming anaerobes, therefore, appears to be both inhibitory and germicidal.

THE EFFECT OF AIR ON THE GASSY FERMENTATION IN SWISS CHEESE

Several experiments were made to determine what effect running air itself through the milk would have on the gassy fermentation. The results of the following experiment are shown in table 4: In cheeses 1 and 2 air was run into milk by means of a copper coil; in nos. 3, 4, and 5 the air was forced through the milk by means of an especially constructed aerator, the purpose of which was to insure a maximum aeration of the milk. The aerator consisted essentially of a cylinder of two compartments separated from each other by means of a perforated disc of tin

TABLE 4

Effect of air on the gassy fermentation in Swiss cheese

NUMBER	QUANTITY OF MILK	QUANTITY OF GAS CULTURE	MANNER OF AERATION	TEMPERATURE OF MILK TREATED	GRADE OF CHEESE*	EFFECT OF AIR†	REMARKS
	lbs.	*cc.*		*cc.*			
1	370	1,100	Air blown through coil for 20 minutes	2	No. 1	0	More of a pressler tendency than check
1-1‡	370	1,100		2	F. g		Trace gas edge
2	370	1,000	22 cubic feet of air through	6	No. 2	0	Both cheeses pressler and nissler
2-1‡	370	1,000		6	No. 2		
3	370	1,000	Through aerator twice	3	No. 2	0	Both cheeses pressler and nissler
3-1‡	370	1,000		3	No. 2		
4	370		Through aerator once	6	No. 2	0	Very much overset. Not so good as check
4-1‡	370			6	No. 1 p		
5	370		Through aerator once	6	No. 2	0	Genuine nissler
5-1‡	370			6			The better of the two

* F = fancy (Fancy and No. 1 further divided: g = good; f = fair; p = poor).

† 0 = no improvement.

‡ Check cheese.

containing 75 small holes through which the milk slowly percolated from the upper to the lower chamber. Beneath the level of the lower chamber the milk was drawn off by a pipe containing a U-shaped bend. Several inches of free space thus existed between the surface of the milk and the disc. Air was forced into the lower chamber by means of a motor-propelled sirocco fan which caused the air to come in contact with the milk dropping from the upper chamber and then to pass through the perforated disc into the milk in the upper chamber. Gas cultures were not used in cheeses 4 and 5 as the milk was of poor quality.

Experiments show that Swiss cheese made from milk treated with air showed no improvement with respect to checking gassy fermentations.

SUMMARY

Ozone and oxygen had a somewhat similar effect in checking gassy fermentation in Swiss cheese occasioned by the introduction of spore-forming anaerobes and did not interfere with the normal functioning of the ripening agents. Within the limits used the temperature did not seem to influence appreciably the effectiveness of these germicides.

Ozone, however, generally imparted such an objectionable flavor to the cheese as to render it unmarketable.

In the case of oxygen some advantage was observed in introducing 20 cubic feet of the gas instead of 10 cubic feet.

In two cases when oxygen was added to milk delivered once a day and the cheese was made upon a commercial scale without the addition of gas-producing organisms, favorable results were observed in checking nissler tendencies. This suggests, at least for this particular factory, that the organisms responsible for abnormal fermentations were anaerobic in character.

The action of oxygen, as indicated by dilution and flask tests, seemed germicidal as well as inhibitory.

In the case of air the oxygen appeared to be diluted too much to exert any favorable influence upon the gassy fermentation.

Acknowledgment is given to W. R. Albus for furnishing cultures, and to S. A. Hall and Wm. T. Johnson for assistance in trying out the use of oxygen with Swiss cheese on a commercial scale.

THE IMMEDIATE INFLUENCE OF FEEDS UPON THE QUANTITY AND QUALITY OF COW'S MILK

I. THE EFFECT OF GROUND FLAX*

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INTRODUCTION

It is a commonly accepted fact that feeds in general exert no specific effect upon the fat percentage in milk over a long period of time. It is, however, generally agreed that certain feeds will cause a temporary increase in fat percentage which will gradually return to normal even though such feed is continuously administered. Many also believe that certain feeds will stimulate milk production for a short period to a point that cannot be maintained by continuous feeding of such feeds. While these facts are generally known and substantiated by experimental evidence, a search of the literature failed to reveal any evidence as to how soon after the administration of a feed the stimulating effect occurred upon either the quantity or quality of the milk.

If the sudden introduction of a feed into the ration will cause an immediate increase in the fat percentage, or the amount of milk, or both, then it becomes possible to take advantage of this in getting more credit for a cow on semi official test[1] than she actually produced. It is because of this possibility that an investigation was undertaken at the University of Minnesota to determine the immediate effect and the extent to which the quantity and quality of cow's milk might be affected by feeding of certain feeds.

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[1] The present system of semi-official testing consists of a one or two-day test each month by official testing supervisors. The fat percentage found on such days is applied to the monthly milk production for the monthly fat credit. The milk weights found by the official test supervisors serve as a check upon the weights reported by the breeder.

Ground flax, being a feed commonly thought of as being capable of influencing both the quantity and quality of milk, was the first studied, and the results of which study are herein reported.

REVIEW OF LITERATURE

The question of the effect of various fatty feeds upon the quantity and quality of milk is an old one, and was the subject of much of the early investigational work in dairy husbandry. The early investigations were conducted from a standpoint of more or less permanent effect of continuous feeding of fatty feeds upon the quantity and quality of milk. While results of different workers were not uniform, the preponderance of evidence was to the effect that fatty feeds do not permanently affect the fat percentage of milk. Anderson (1) as early as 1899, in reviewing the literature to that time and reporting results of his own investigations, came to that conclusion. Of thirteen investigations reviewed by him, only four were reported as showing an increase in fat due to the feeding of fatty feeds. Morgan and co-workers (2), summarizing the work in 1904, cited four investigators as having secured marked increase in amount of milk and fat percentage; thirteen as having secured only slight increases which were only temporary; and three as having secured no effects from the feeding of fatty foods.

That some investigators secured positive results and others negative results is explained by Stohman and co-workers (3) as being due to the fact that individuality of the animals determines whether or not they will respond. To this may be added that the short experimental periods usually used in the reversal type of experiment would indicate temporary effects as being permanent.

While no investigations reported showed the immediate influence, it is of interest to find that different investigators reported an increase in fat percentage or amount of milk or both as a result of feeding flax or flax products. Lindsey (4), feeding 1.4 pounds digestible oil, daily, in ground flax, increased the fat percentage from 5.0 to 5.56 per cent, which gradually returned to normal in four to five weeks. Einecke (5), working with goats, secured an increase in milk flow in all cases when 50 grams linseed oil were fed daily, and an increase with some animals and a decrease with others when 30 grams were fed. In all cases the fat percentage rose. Beglarian (6), feeding ground flax observed an increase in the amount of milk but no influence on the fat percentage, with four cows on four experiments of eight days each. Morgan and co-workers (7), feeding 1 gram linseed oil per kg. live weight to two goats and eight sheep reported various results from a decrease in both milk and fat to a very marked increase.

This brief review of the extended literature on the subject is sufficient to show that marked permanent effects upon either the quantity or quality of the milk is not secured by the feeding of flax or flax products but that the temporary effects have often been noted.

THE PROBLEM

As it is fairly well established that no feed exerts a specific influence upon the fat percentage over a long period of time, no attempt was made in this investigation to ascertain over how long a period of time ground flax might exert an influence. This investigation is concerned primarily with the possibility of ground flax exerting a specific influence upon milk or milk-fat production of cows immediately after ingestion. From this standpoint, the following factors were considered:

1. The effect upon the fat percentage and amount of milk.
2. Uniformity of response from time to time.
3. Individual variations. Probable causes and factors that might be correlated with them.
4. Significance of results as applied to semi-official testing.

PLAN OF THE EXPERIMENT

All cows on official test at University Farm during the calendar year were included in the experiment. The official inspection covering two days, with a preliminary milking each month, was conducted in the usual way by a regular official test supervisor. The flax feed period consisted of the forty-eight-hour period immediately following the regular official inspection period.

The tests of the flax feed period were conducted in the same manner and by the same supervisor as official test period.

The ration for the two periods was the same except that on the last feed of the official test period and for each feed of the flax period, one pound of ground flax was substituted for an equal amount of the regular grain mixture. In all other respects the handling and care of the cows for the two periods was identical.

The cows were milked three times daily until the production dropped to the point where the University Farm scale called for a change to two-time milking. Thus when milked three times daily, each cow received three pounds ground flax, and when milked twice daily, each cow received two pounds flax daily.

TABLE 1

The effect of feeding ground flax upon the fat percentage in milk

COW NUMBER	BREED	NUMBER OF TEST PERIODS	AVERAGE FAT PERCENTAGE		
			Normal period	Flax period	Increase
152	Jersey	12	5.382	5.671	+0.289
144	Jersey	9	5.239	5.484	+0.245
145	Jersey	7	4.621	4.636	+0.015
140	Jersey	7	5.791	5.844	+0.053
141	Jersey	5	6.630	6.831	+0.201
143	Jersey	8	6.298	6.464	+0.166
360	Holstein	9	2.663	2.654	−0.009
376	Holstein	8	3.043	3.087	+0.044
350	Holstein	10	3.058	3.068	+0.010
361	Holstein	7	3.480	3.465	−0.015
355	Holstein	9	2.959	3.134	+0.175
358	Holstein	8	3.320	3.555	+0.235
520	Guernsey	7	5.222	5.250	+0.030
536	Guernsey	6	4.567	4.909	+0.342
526	Guernsey	8	4.837	4.971	+0.134
527	Guernsey	4	5.179	5.368	+0.189
Average			4.518	4.649	+0.131

All cows were fed alfalfa hay, corn silage, beet pulp, and the University Farm test ration consisting of:

Corn	**100 parts**
Bran	**100 parts**

Ground oats	100 parts
Barley	100 parts
Linseed oil meal	100 parts
Cottonseed meal	50 parts
Corn gluten	50 parts
Salt	9 parts

DISCUSSION OF RESULTS

General effect upon milk and fat percentage. From table 1 it will be seen that the average fat percentage for the 16 cows is 0.131 points or 2.9 per cent higher for the two-day flax periods than for the normal feed period. Further analysis reveals that 14 out of the 16 cows increased, and only two decreased the fat percentage when fed ground flax. However, for the two that decreased and four of those that increased the fat percentage, the variation is less than one per cent from the normal feed period. For the rest the effect is appreciable, varying from increases of

TABLE 2

The effect of feeding ground flax upon the two-day milk and fat yield

COW NUMBER	AVERAGE TWO-DAY MILK YIELD			AVERAGE TWO-DAY FAT YIELD		
	Normal period	Flax period	Increase	Normal period	Flax period	Increase
152	52.9	54.3	+1.4	2.86	3.08	+0.23
144	44.3	46.4	+2.1	2.32	2.54	+0.12
145	46.5	48.0	+1.5	2.15	2.22	+0.07
140	36.4	36.1	−0.3	2.11	2.11	0.00
141	16.8	18.3	+1.5	1.11	1.25	+0.14
143	28.9	30.2	+1.3	1.82	1.95	+0.13
360	106.0	108.7	+2.7	2.83	2.89	+0.06
376	115.7	117.6	+1.9	3.52	3.63	0.11
350	93.5	90.1	−3.4	2.86	2.77	−0.09
361	74.0	74.9	+0.9	2.57	2.60	+0.03
355	90.3	87.8	−2.5	2.67	2.75	+0.08
358	80.0	79.8	−0.2	2.66	2.84	+0.18
520	35.9	37.2	+1.3	1.87	1.95	+0.08
536	69.4	70.5	+1.1	3.17	3.46	+0.29
526	51.3	52.8	+1.5	2.48	2.62	+0.14
527	48.0	48.4	+0.4	2.49	2.60	+0.11
Average	61.9	62.6	+0.7	2.47	2.58	+0.11

1.5 per cent to 7.1 per cent. In four cases the actual increases in fat percentage are more than 0.2, and in one case it is 0.342.

From table 2 it will be seen that on the average the milk production for the two-day flax period is 0.7 pounds or 1.1 per cent more than for the two-day normal feed period. Further study of this table reveals that different cows responded in widely varying degrees. Four cows declined and twelve cows increased in milk production when fed ground flax. The variation is from a decline of 3.6 per cent to an increase of 8.9 per cent. In six cases the increase or decrease is less than 1 per cent from normal, and may be considered as insignificant.

Table 2 also shows the influence of ground flax on the fat percentage and milk combined, and expressed as total fat production for the two-day period. Fourteen cows responded to the feeding of ground flax by increases in fat production varying from 0.9 to 12.1 per cent over the normal feed period. In one case there will be noted a decrease of 0.90 pounds fat or 3 per cent, and in the other case, no effect. The decrease in fat production is due to the depressing influence of flax upon milk flow as there is no appreciable influence upon the fat percentage in this case. Cow 536 was habitually low on the first milking of the official test which was with two exceptions the evening milking, and highest on the second or morning milking. It will be noted that on the flax period both the milk and the fat percentage is higher for the first milking and lower for the second milking than for the normal feed period. After the second milking the fat percentage gradually increases reaching the maximum at the fourth milking.

Figure 1 illustrates graphically the immediate effect of ground flax upon the milk production and fat percentage for three cows. It shows the average amount of milk and fat percentage for each of the six milkings during the experiment for both the normal feed and flax periods. Cow 152 increases both milk production and fat percentage when fed flax. The stimulating effect upon milk production was immediate as the first milking of the flax period was the highest which was eight hours after the first feeding of flax. It will also be noted that the curve for milk production is uniform up to the sixth milking when it drops off abruptly. Fat percentage is also increased on the first milking but there is a gradual rise up to the fifth milking when the maximum is reached.

Uniformity of response. Figures 2, 3, and 4 illustrate what may be expected in the way of difference of response by different cows from month to month. Figure 2 is the graph, by months, of the production record of cow 152 for both normal feed and flax periods. It shows that both the amount of milk and the fat percentage was stimulated fairly uniformly with the exception of the first, tenth and eleventh months of the lactation period. The increase in fat percentage and the decrease in amount of milk during the flax period of the first month may be attributed

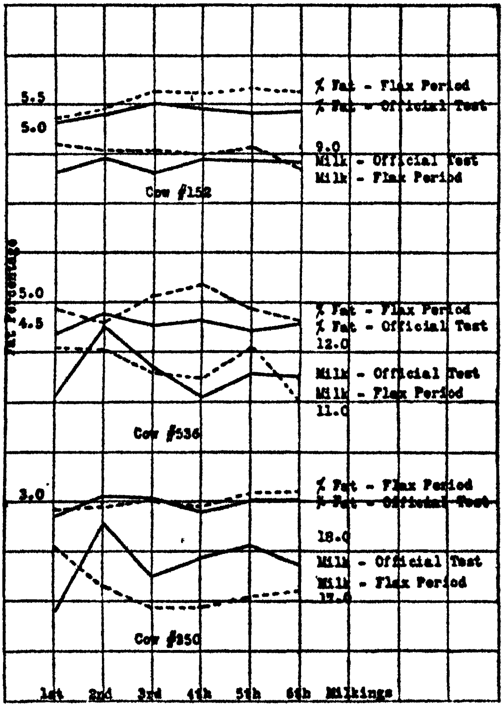

FIG. 1. SHOWING THE IMMEDIATE EFFECT OF GROUND FLAX UPON FAT PERCENTAGE AND AMOUNT OF MILK ON THREE COWS

The corresponding milkings of the flax feed period and the normal feed period are compared.

to the fact that she was in oestrum during the flax period. The higher fat percentages for the official test period of the tenth and eleventh month is unaccounted for. It may be noted, however, that the fat percentages for these months are unusually high on the official test period and that the flax period presents a smoother curve.

Cow 350 showed no response to feeding of ground flax as far as fat percentage is concerned but showed a rapid decline in milk, reaching the minimum at the third milking. For some unknown reason, this cow averaged low on the first milking of the normal feed period and high on the second milking. In the other cases where ground flax had a depressing influence upon milk production, it stimulated fat percentage sufficiently to account for an increase in total fat production.

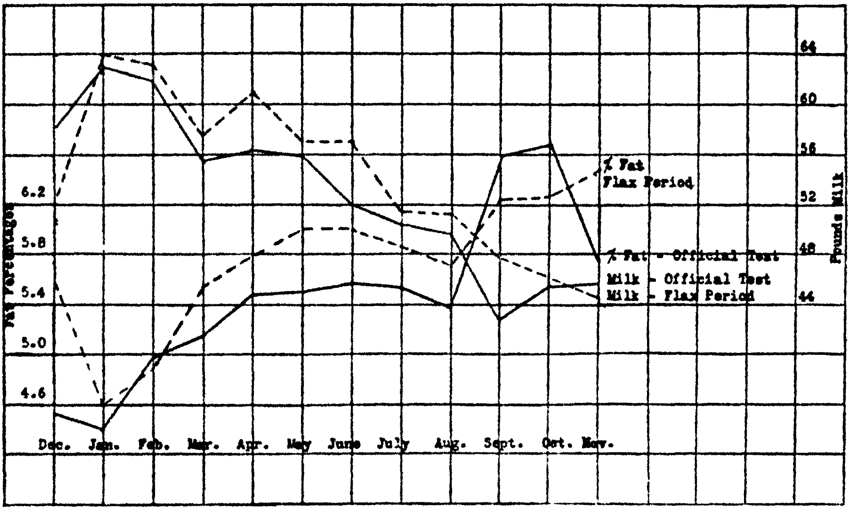

Fig. 2. Comparison by Months of Fat Percentage and Amount of Milk for Flax Period and Normal Feed Period for Cow 152

Figure 3 illustrates the uniformity of response to the depressing influence of ground flax upon the milk production of cow 350, and varied influence upon the fat percentage. With the exception of one month, the milk production was lower each time for the flax period than for the official test period.

Figure 4 illustrates a variance in response to the feeding of flax both for milk production and fat percentages. On the average flax caused a slight increase in both milk production and fat percentage but a variable response from month to month.

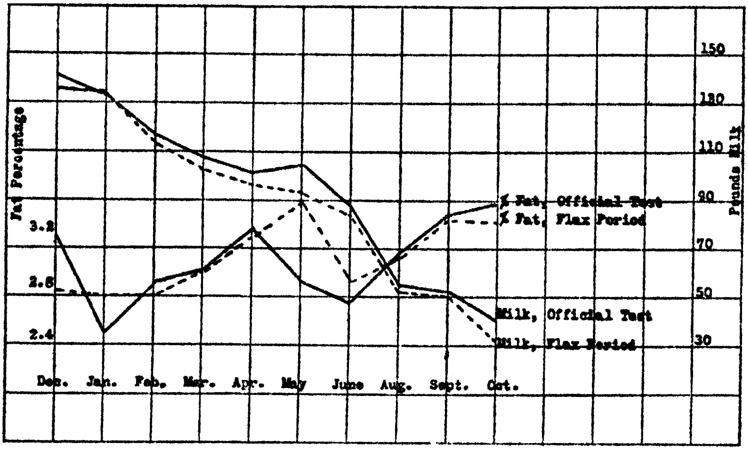

FIG. 3. COMPARISON BY MONTHS OF FAT PERCENTAGE AND AMOUNT OF MILK FOR FLAX PERIOD AND NORMAL FEED PERIOD OF COW 350

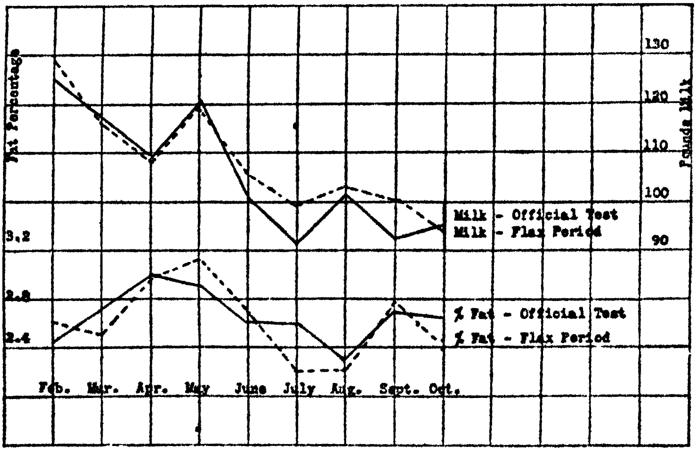

FIG. 4. COMPARISON BY MONTHS OF FAT PERCENTAGE AND AMOUNT OF MILK FOR FLAX PERIOD AND NORMAL FEED PERIOD FOR COW 360

Individual variations and probable causes. One of the significant results of this investigation is that individual animals vary in the way and degree that they respond to the feeding of ground flax. From the data herein presented, we would not be warranted in concluding that ground flax has a depressing effect upon the fat percentage of some cows but can conclude that with some it has no effect and that with others the fat percentage is stimulated to varying degrees. With milk production, the feeding of ground flax may have a depressing effect on some cows, no influence on others, and a stimulating influence on still others. Wherever there was either a marked depressing or stimulating effect on either the average milk or fat production, such influence was uniform each time the animal was fed ground flax.

Neither stimulating nor depressing influences of flax feeding could be correlated with breed, capacity for milk production, normal fat percentage of milk, season of year or period of the lactation. As to whether or not an animal will respond by an increase or decrease in either the amount or the fat percentage of milk through the feeding of ground flax depends upon some unknown factor peculiar to the individual. From the data herein presented, it would appear that separate factors are involved for milk and fat percentage as a depressing effect upon milk production may or may not be accompanied by a stimulating effect upon fat percentage or vice versa.

As an increase in body temperature is usually believed to cause an increase in the fat percentage of the milk, it was thought possible that the ingestion of the relatively large amounts of ground flax might cause metabolic disturbances that in turn might increase the body temperature. Temperatures were then taken for three consecutive months at eight hour intervals for both normal and flax feed periods. When analysis of the data revealed no increases in body temperature from the feeding of flax, this particular phase of the work was discontinued.

Significance of results as applied to semi-official testing. As the average fat percentage credited a cow on semi-official test depends upon the results secured by the official test supervisor on the monthly one or two-day inspections, it is evident that anything that will immediately and temporarily increase the test will credit the cow with more butterfat than she is entitled to. If milk production can be consistently increased by the specific effect of a feed during the one or two-day official monthly inspection, the owner can pad the milk reports so as to get more credit than a cow is entitled to, and such padding cannot be detected from inspection of the milk reports. Table 3 shows that by taking advantage of the temporary stimulating effects of ground flax upon both the amount and fat percentage of milk 14 out of 16 cows would have been credited with more than their actual butterfat production, and only one would have received less credit. The increases in total butterfat production varied from 0.9 per cent to 12.1 per cent, while the one decrease amounted to 3 per cent.

TABLE 3

The effect on official test record of applying flax period fat percentage and milk yield during experimental period

COW NUMBER	INCREASE OF OFFICIAL TEST BUTTERFAT RECORD MADE POSSIBLE BY APPLICATION OF:			
	Flax period fat percentage		Combined milk and fat percentage	
	pounds	*per cent*	*pounds*	*per cent*
152	24.66	4.6	43.42	8.1
144	13.21	3.9	17.68	5.3
145	−0.10	0.0	8.75	3.6
140	2.16	0.9	0.00	0.0
141	2.44	2.9	10.03	12.1
143	6.78	3.0	16.46	7.3
360	−0.50	−0.1	7.82	2.0
376	7.44	1.3	12.99	3.0
350	0.84	0.0	−13.18	−3.0
361	1.63	0.6	2.29	0.9
355	17.72	4.7	9.65	2.6
358	24.04	7.6	21.68	6.8
520	2.17	1.0	8.58	4.3
536	22.27	7.2	28.28	9.2
526	8.35	2.7	16.95	5.7
527	8.01	5.0	7.23	4.5
Average....................		2.8		4.5

Cow 152 was credited officially with 536.4 pounds fat. Had advantage been taken of the stimulation caused by the feeding of ground flax, and the results secured on the flax periods applied instead of the official tests, she would have been credited with 579.8 pounds fat during the same period. Calculated on this same basis, cow 536 shows an increase of 28.28 pounds fat for a period of six months.

With some cows, therefore, advantage can be taken of the stimulating effect of ground flax to secure more credit than is actually due on semi-official test. However, the owner must know how the cow is going to respond in order to do this as the irregularity of response of some cows from month to month will often give the actual milk report the appearance of having been padded when ground flax is fed for the official test period.

CONCLUSIONS

From the results of this investigation it may be concluded that:

1. The response of animals to the feeding of ground flax depends upon unknown individual factors. Different animals respond in different ways and to different degrees, and such differences are not correlated with season of the year, breed, amount of yearly milk production or normal fat percentage of the milk.
2. When ground flax is fed at the rate of three pounds daily the majority of cows will show an increase in fat percentage. Such increases ranged from no effect to 7.1 per cent and averaged 2.9 per cent.
3. The slight decrease in fat percentage in two cases out of sixteen cows will not warrant the conclusion that ground flax may cause a decrease in fat percentage but rather that in some cases it has no appreciable effect.
4. The feeding of ground flax will in the majority of cases cause an increase in amount of milk averaging 1.1 per cent but going up to 8.9 per cent, but ground flax may also have a depressing influence upon the milk production with a few cows.
5. On the average the combined influence of flax upon amount of milk and fat percentage increased the total fat production 4.5 per cent. This influence varied from a decrease of 3.0 per cent to an increase of over 12.0 per cent.

6. With cows showing appreciable responses, on the average, the response was uniform from month to month, making it possible to secure more than actual credit for cows on official test.

7. The response, whether an increase or decrease, was immediate with most cows, appearing in most cases within eight hours after the first feeding. This makes detection of such feeding practices impossible from inspection of official test record.

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DAIRY NOTES

ADULTERATION OF MILK

In most of the books which treat of the subject, the formulae given for calculation of the percentages of added water and fat removed in cases of adulterated milk are as follows:

$$p = 100(1 - f/F); \; w = 100(1 - n/N)$$

f and n are respectively the percentages of fat and non-fatty solids found, F and N the percentages known or assumed to have been originally present, w the percentage of added water and p the percentage of the fat removed.

The first of these two formulae is not exact; and should be:

$$p = 100\left(1 - \frac{f}{F}\right) \times \frac{100}{100 - f}$$

In any case it fails entirely if the milk has been watered. The second is exact only if none of the fat has been removed. This is generally recognised and some of the books give other formulae to be used in special cases, i.e., when the milk has been both skimmed and watered.

These special formulae are not regarded with much favour by analysts. In some cases they are too complicated; in others they are obviously inaccurate. If it were exact, the formula for fat removed should give the true result whether the amount of added water were large or small or even if it were zero. Similarly, the formula for added water would be independent of the amount of fat removed.

In other words, if they were exact, these formulae for the special case of milk that has been both skimmed and watered would be perfectly general; they would be applicable in every case and no others would be required. It is the purpose of this article to show that such expressions can be found, that they are quite simple and that those previously referred to can therefore be dispensed with. The method by which they are derived is briefly outlined below.

If 100 grams of milk contain 4 grams of fat and 9 grams of non-fatty solids and 100 grams of water be added to it there will be 200 grams of mixture. This will contain 50 per cent of added water and the original amounts, but only half the percentages, of the other ingredients. If now 1 gram (25 per cent) of the fat be removed there will be left 199 grams of skimmed and watered milk and this will contain 3 grams (= 1.5075 per cent) of fat and 9 grams (= 4.5226 per cent) of non-fatty solids.

In general, if f' and n' be the percentages of fat and non-fatty solids, respectively, in the skimmed and watered milk and the other symbols as before, then:

$$f = \frac{F(100 - w)}{100}; \; f' = \frac{100f(100 - p)}{10{,}000 - pF}$$

$$n = \frac{N(100 - w)}{100}; \; n' = \frac{10{,}000n}{10{,}000 - pf}$$

Substituting the values of f and n in the second equation in each case this gives:

$$f' = \frac{100F(100 - w)\,(100 - p)}{1{,}000{,}000 - pF(100 - w)}; \; n' = \frac{10{,}000N(100 - w)}{1{,}000{,}000 - pF(100 - w)}$$

As each of these equations involves both p and w, these factors cannot be evaluated from either; but if they are treated as a pair of simultaneous equations, expressions can be found for both p and w. The result is as follows:

$$p = 100\left\{1 - \frac{Nf'}{n'F}\right\}; \; w = 100\left\{1 - \frac{100n'}{N(100 - f') + n'F}\right\}$$

If 3 per cent of fat and 8.5 per cent of non-fatty solids be taken as the original amounts of these ingredients and f and n be the percentages found, the formulae become:

$$p = 100\left\{1 - \frac{8.5f}{3n}\right\}; \; w = 100\left\{1 - \frac{100n}{8.5(100 - f) + 3n}\right\}$$

These formulae are not unduly complicated. They are exact and are therefore applicable whether the milk is both skimmed and watered or adulterated in one way only. The formula for added water is new; at all events the author has not seen it given previously.

The formula for fat removed is not new; it was given by Bohmlander (Chemiker-Zeitung, vol. xvii) in 1893. Exception may be taken to it on the ground that it is based on the alternation of the ratio F/N, which of course is not affected by addition of water, and that the legislation under which action is taken in such cases does not mention and therefore does not sanction the use of this or any other "ratio."

It is evident, however, from the manner in which the formula is derived that it is a legitimate mathematical inference from the given premises. It is therefore, in that respect, just as valid as the formula for fat removed which is applicable only when it is known or assumed that no water has been added. In fact, under these circumstances, it gives the same result.

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THE RELATION OF SUNLIGHT TO THE GROWTH AND DEVELOPMENT OF CALVES*

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In recent years the importance of sunlight to the health and well-being of the human infant has been fully established. Evidence has also accumulated which indicates that it bears a similar relation to at least some of the domestic animals. Thus it has been shown that poultry, especially baby chicks, are very susceptible to the absence of sunlight which apparently acts as a supplement to, or the equivalent of, the antirachitic factor of foodstuffs (1) (2). Swine also exhibit a need for it, (3) in fact gross symptoms as well as chemical analysis of blood and bone, together with histological examinations have indicated that sunlight is a factor of economic importance in the production of pork under confined conditions (4). In the case of the bovine, Hart and Steenbock (5) with mature milking cows obtained an increase in the amount of calcium retained by animals exposed to sunlight as compared with those kept in the dark, but this difference was less than had been expected. The greater susceptibility of the young growing animals of other species to a lack of sunlight, however, suggests the probability of a similar tendency in the young bovine. As yet no study has been reported of the relation of this factor to the development of calves. There is great need, however, for such information in connection with the correct interpretation of results from experiments in which calves are used and kept under confined conditions.

EXPERIMENTAL

According to the original plan, the experiment was to have terminated when the calves were six months old, the object of

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the experiment being to observe the effect of a lack of sunlight upon the growth and well-being of young calves, but when at that age the results were all negative, it was decided to extend the period to include the time of first freshening or until the animals were about two years old. This made it possible to observe the effect of the absence of sunlight on the growth and well-being of the calves over a longer period and in addition also indicated, to a limited extent, its influence on reproduction.

Animals used and treatment

Four grade Holstein heifer calves designated by the numbers E-48, E-50, E-51 and E-52 respectively were used in the experiment. These animals were purchased in June, 1924, when from three to seven days old, and were from dams that had been kept under average farm conditions. Two of the calves, E-48 and E-50, known as the "no sunlight" group, were placed immediately on their arrival, in completely dark box stalls and thereafter during the period of the experiment were never exposed to direct sunlight. The stalls were of ample size to allow considerable exercising, and excellent ventilation was provided by means of a large electric fan. Occasionally, they were allowed to run free in the experimental yard out of doors during the dark part of the night. The other two calves, E-51 and E-52, known as the "sunlight group" were also kept in box stalls of the same size and shape as those provided for the others, but with no restrictions on the amount of light. During the first six months the practice was followed of placing them in pens of the same size out of doors in direct sunlight. After that, they were allowed to run freely with other experimental calves during the day time, being confined in their stalls in the barn at night. This did not decrease their exposure to sunlight, but no doubt increased the amount of exercise taken.

Ration fed

The ration, which was uniform for the four animals, was not selected as ideal for dairy calves, but because it is typical of that fed on many dairy farms, and is also rather low in calcium which should make conditions favorable for positive results if sunlight is a factor in the utilization of this mineral by cattle. Whole milk was fed during the first three or four weeks, then replaced with skimmilk which was fed until the calves were about six months old. Timothy hay of fair to poor quality, fed ad libitum, constituted the only form of roughage. The grain fed consisted of a mixture of three parts by weight of cornmeal and one part each of corn gluten feed, wheat bran, and linseed oilmeal. During the first few months grain was fed ad libitum, but after that the amount provided was regulated so as to furnish the nutrients required according to the Morrison standard for growing dairy cattle. Shavings were used for bedding, so no nutrients were obtained from that source.

Weights of the animals were taken every ten days and measurements of height at withers were made every thirty days. Daily observations were made of the condition and behavior of all animals and any abnormality noted.

RESULTS

Throughout the entire period of approximately two years, all four animals continued normal in all outward respects. As is shown in figure 1, with few exceptions, all made gains equal to or better than normal during the entire period. It is worthy of note, however, that from the beginning almost invariably the two calves raised in the dark made better gains than did the check animals. This was, perhaps, to be expected if the lack of sunlight did not act as too great a disturbing factor, because these animals were more comfortable and received less exercise than the others that were turned out of doors. Table 1 shows that the ration was ample both as to protein and total digestible nutrients, usually supplying a slight excess of both over the amounts prescribed by the Morrison standard.

All the heifers came in heat the first time when less than a year old and the two check animals, E-51 and E-52 were bred and became pregnant at their first heat period due to the presence of

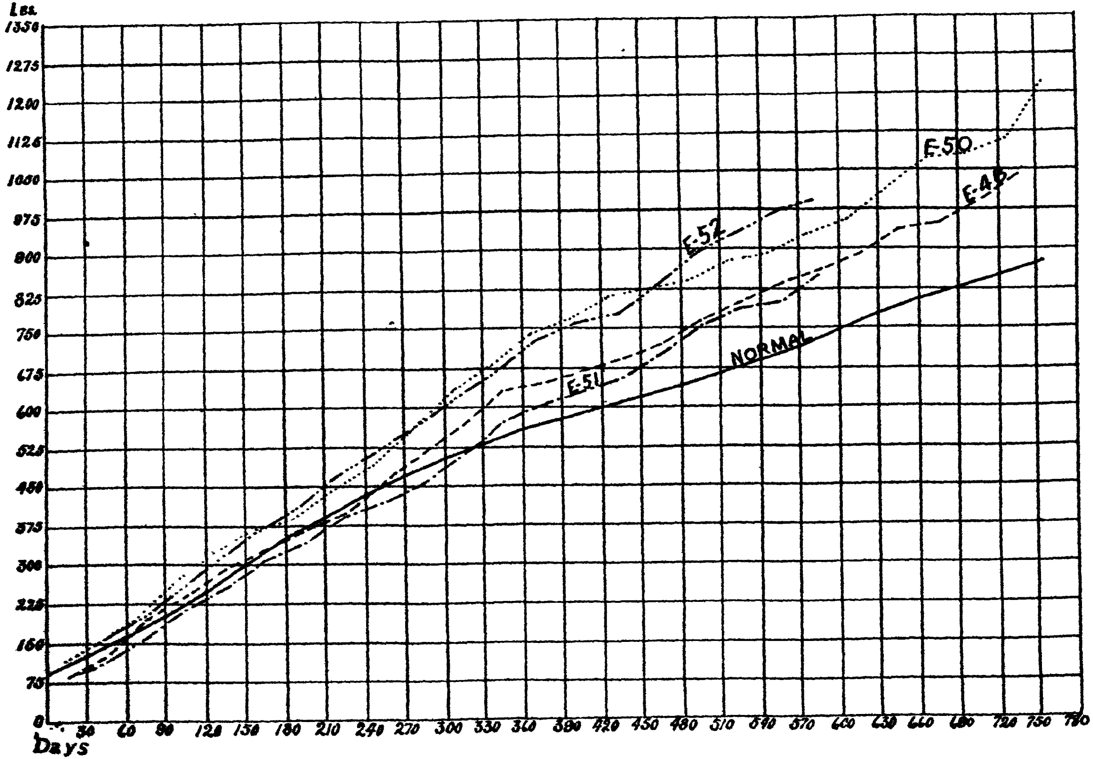

FIG. 1. GROWTH OF THE FOUR CALVES USED IN THE EXPERIMENT COMPARED WITH THE NORMAL CURVE All received similar rations but E-48 and E-50 received no sunlight during the entire period.

TABLE 1

Showing daily rations fed and nutrients and minerals provided each calf at intervals of 120 days beginning at 30 days of age, also nutrients required by the animals at the different ages according to Morrison standard

NUMBER OF ANIMAL	GROUP	AGE	DAILY RATION		NUTRIENTS IN RATION		NUTRIENTS REQUIRED BY ANIMAL (MORRISON STANDARD)		MINERALS IN RATION	
			Timothy hay	Grain§	Crude protein	Total digestible nutrients	Crude protein	Total digestible nutrients	Calcium	Phosphorus
		days	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*grams*	*grams*
E-48	No sunlight	30*	0 5	0 5	0 43	2 42	0 33	1 97	5 23	4 88
		150†	2 0	4 5	1 32	5 67	0 73	4 93	12 25	18 21
		270	5 5	6 0	1 10	7 46	0 94	7 22	6 98	19 21
		390	10 0	6 0	1 14	9 55	1 14	8 90	10 63	21 97
		510	14 0	6 0	1 33	11 52	1 26	9 76	13 87	24 43
		630	14 0	6 0	1 28	11 76	1 36	10 50	13 87	24 43
		740	13 0	8 0	1 55	12 77	1 44	10 96	13 90	29 09
E-50	No sunlight	30*	0 5	0 5	0 43	2 42	0 40	2 48	5 23	4 88
		150‡	3 0	5 0	1 51	6 74	0 81	5 65	14 51	20 90
		270	6 0	7 0	1 27	8 49	1 00	7 79	7 80	22 15
		390	12 0	7 0	1 34	11 31	1 24	9 61	12 67	25 84
		510	14 0	7 0	1 49	12 28	1 33	10 33	14 29	27 07
		630	16 0	7 0	1 48	13 55	1 41	10 85	15 91	28 30
		750	17 0	9 0	1 81	15 59	1 56	11 38	17 56	34 19
E-51	Sunlight	30*	0 5	0 5	0 43	2 42	0 31	1 80	5 23	4 88
		150†	2 0	4 5	1 32	5 67	0 70	4 69	12 25	18 21
		270	5 5	6 0	1 10	7 46	0 89	6 62	6 98	19 21
		390	10 0	6 0	1 14	9 55	1 09	8 50	10 63	21 97
		510	16 0	6 0	1 39	12 50	1 25	9 68	15 49	25 66
		600	16 0	6 0	1 34	12 78	1 32	10 23	15 49	25 66
E-52	Sunlight	30*	0 5	0 5	0 43	2 42	0 39	2 39	5 23	4 88
		150‡	3 0	5 0	1 51	6 74	0 79	5 54	14 51	20 90
		270	6 0	7 0	1 27	8 49	1 02	7 95	7 80	22 15
		390	12 0	7 0	1 34	11 31	1 22	9 48	12 67	25 84
		510	14 0	7 0	1 49	12 28	1 34	10 38	14 29	27 07
		600	16 0	7 0	1 48	13 55	1 27	9 89	15 91	28 30

* Ration also included 10 pounds whole milk.

† Ration also included 14 pounds skimmilk.

‡ Ration also included 16 pounds skimmilk.

§ Grain mixture made up of: 300 cornmeal; 100 corn gluten feed; 100 wheat bran; 100 linseed oilmeal.

a young bull in the experimental lot. E-50, on the other hand, was not bred until she was about sixteen months old when one mating was sufficient for conception. E-48, the other no-sunlight heifer, was found after being on the experiment for about a year, to have malformed reproductive organs which made it impossible for her to reproduce, although oestrum was possible. This deformity, however, was declared as not being due to the treatment she had received. From table 2 it can be seen that the other three heifers carried their calf the full normal period. All the calves except that of E-52 were normal at birth and all the heifers passed the afterbirth within twenty-four hours after freshening. As indicated in table 2, E-52 of the sunlight group, dropped a rather small and slightly deformed calf, and although both its eyes were present it was entirely blind. It is possible that the ration fed was lacking in the fat soluble vitamin and thus contributed toward

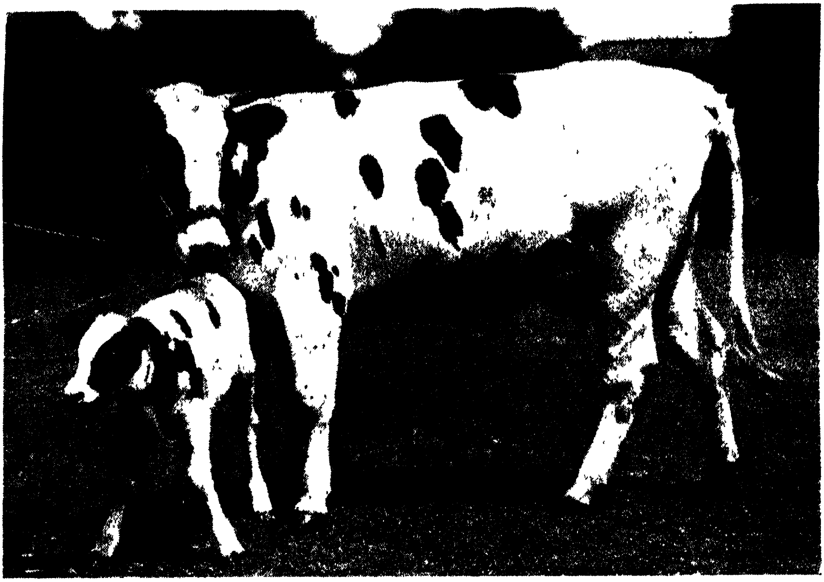

FIG. 2. HEIFER E-50 WITH HER CALF

The calf was dropped after the mother had been kept in the dark from the time she was one week old, a period of over two years. Both animals appear normal in all respects.

this condition. This, however, does not seem probable as both of the other heifers dropped normal calves. It is also well-known that cows kept under normal conditions will sometimes drop deformed as well as blind calves.

The ration, as has already been mentioned, provided an adequate supply of protein and total digestible nutrients as based on the Morrison standard, but was, as is shown in table 1 exceedingly low in its calcium content; in fact, when considered on the dry matter basis it contained only about 0.2 per cent of calcium. The significance of this fact is apparent when, according to Meigs and his coworkers, it is considered that perhaps only about half of the total amount of calcium present in the ration is assimilated. The supply of available calcium was sufficient, however, to enable all four heifers to make better than normal gains in both weight and height and to continue in all outward respects in normal well-being during the entire period of the experiment. The fact that no significant differences were noted between the two groups at any time appears to indicate that the lack of sunlight did not exert any considerable effect on calcium assimilation, otherwise it seems it would have become apparent during the two year period when the calcium supply was at such a low level.

TABLE 2

NUMBER OF ANIMAL	GROUP	GESTATION PERIOD	SEX OF CALF	WEIGHT OF CALF	CONDITION OF CALF AT BIRTH
				pounds	
E-48	No sunlight	Deformed	Reproductive organs		
E-50	No sunlight	Normal	Male	89	Normal
E-51	Sunlight	Normal	Female	79	Normal
E-52	Sunlight	Normal	Female	60	Blind and slightly deformed

Another matter worthy of notice in connection with the mineral content of the ration is the relative amounts of calcium and phosphorus present. As is shown in table 1, the total amount of phosphorus almost invariably exceeded that of the calcium, and most of the time by nearly two to one. This represents a condition almost the opposite of the optimum as recommended by McCollum (6, 7) and according to his views (8) "the ratio between the concentration of calcium and of phosphorus in the diet may, within certain limits, be of greater significance to the welfare of an animal than the absolute amounts of these substances which the diet contains." The fact, however, that no ill effects were noted in any of the animals would indicate the condition was not a serious disturbing factor in either of the groups.

CONCLUSION

So far as could be measured by general observations, weights, and rate of skeletal growth, the absence of sunlight was without effect upon calves kept in darkness from the age of one week to two years. Normal reproduction also occurred.

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PERSISTENCY OF FAT SECRETION DURING THE LACTATION PERIOD AS AFFECTED BY AGE*

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In a previous paper (1) quantitative forms of expressing the persistency of milk and fat secretion during the lactation period were described. It was shown that when all conditions are held as uniform as is possible, the monthly milk or fat production during the lactation period after the maximum is passed, is a constant percentage of the preceding month's production. Pregnancy, seasonal changes (especially environmental temperature), the state of nutrition, and management of dairy cows may, however, cause slight changes in the rate of decline of milk and fat secretion.

In a study of the inheritance of the "persistency character" in the Guernsey breed, it was suggested that age might be an important factor influencing the persistency of milk and fat secretion. The only information found on the subject was the data presented by Sanders (2) of Cambridge, in a study of the lactation curve of English milk records. He determined the ratio of the total lactation yield to the maximum daily yield, which he called the *shape figure* (S.F.) of the cows' lactation. It was shown that the two-year-old animals had a higher S.F. than did the older animals. Since compiling and studying the data later to be presented, a paper by Gaines and Davidson (3) of Illinois, has appeared in which the statement is made that "the value of k tends to increase with age and yield; that is, the younger cows are more "persistent" than the older cows."

It appears evident that the age of the cow does influence her persistency of secretion. However, no theory is advanced in either paper to attempt to explain the results obtained. The object of this paper in addition to presenting data showing the

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relation between the persistency of fat secretion during the lactation period and age is to offer a possible explanation for the results.

In the study of persistency of secretion, it is necessary to have the itemized monthly records of production. The Advanced Register of Guernsey cattle is especially valuable as it contains a large number of such records and is the source of the data here presented. Only non-pregnant cows were included in this study as it has been shown that pregnancy affects the lactation curve when it extends more than four months during the lactation period. In order to make the results in each age group more nearly comparable, 100 records in each age group which were satisfactory in every respect were used. This was considered preferable to including a large number of records in the immature classes and only a limited number in the classes at advanced

The influence of all other factors which might affect persistency have been neglected. It was hoped that the number of records would be sufficient to provide a random sample which would minimize the influence of these factors. The average monthly fat production from the second to twelfth months inclusive were determined by yearly age groups beginning at two years.

The results are presented in table 1. The average fat production from the second month to the twelfth month is given. The per cent of the previous month's fat production is calculated in order to determine the rate of decline or persistency of secretion during the laction period. The *average* per cent decline of the previous month's production is considered the best indication of persistency. It will be noted that the average persistency of fat secretion declines rapidly between two and three, years and then less rapidly as maturity is reached. It will be seen that above six years, the number of records available decreases very rapidly, so that the data is unreliable in giving an indication of the effect of age on persistency during the declining segment of the curves of milk secretion, i.e., after eight years. The data by age groups is plotted in figure 1, and a curve drawn through the points. There is some irregularity between the observed values and the

TABLE 1

The effect of age on the persistency of fat secretion

AGE	2 to 3 years		3 to 4 years		4 to 5 years		5 to 6 years		6 to 7 years		7 to 8 years		8 to 9 years	
Number of records	100		100		100		100		100		71		34	
Month of lactation	Average fat	Per cent of previous month's fat production	Average fat	Per cent of previous month's fat production	Average fat	Per cent of previous month's fat production	Average fat	Per cent of previous month's fat production	Average fat	Per cent of previous month's fat production	Average fat	Per cent of previous month's fat production	Average fat	Per cent of previous month's fat production
	pounds		*pounds*		*pounds*		*pounds*		*pounds*		*pounds*		*pounds*	
Second	40.44		47.37		53.94		54.96		58.17		55.49		58.05	
Third	40.92	101.18	46.17	97.46	51.24	94.99	53.52	97.37	55.62	95.61	54.59	98.37	58.14	100.15
Fourth	40.11	98.02	44.13	95.58	50.01	97.59	51.69	96.58	51.45	92.50	51.18	93.75	56.82	97.72
Fifth	40.05	99.85	41.13	93.20	48.21	96.40	49.71	96.16	50.88	98.89	49.22	96.17	52.26	91.97
Sixth	39.18	97.82	40.83	99.27	47.13	97.75	47.70	95.95	48.45	95.22	47.70	96.91	52.05	99.59
Seventh	37.86	96.62	38.97	95.44	45.21	95.92	45.84	96.10	47.04	97.08	45.80	96.01	48.97	94.08
Eighth	37.50	99.04	37.44	96.07	42.78	94.62	43.92	95.81	44.13	93.81	43.80	95.63	45.52	92.95
Ninth	36.52	97.38	36.42	97.27	42.30	98.87	42.15	95.96	43.18	97.84	41.07	93.76	42.35	93.03
Tenth	35.42	96.98	35.22	96.70	40.44	95.60	40.08	95.08	40.12	92.91	39.38	95.88	42.44	100.21
Eleventh	34.46	97.28	33.45	94.97	38.71	95.72	38.07	94.98	38.52	96.01	36.71	93.21	39.97	94.18
Twelfth	33.39	96.89	32.76	97.93	37.84	97.75	36.03	94.64	36.42	94.54	35.49	96.67	36.79	92.04
Average	37.80	98.11	39.44	96.39	45.25	96.52	45.78	95.86	46.73	95.44	45.49	95.64	48.48	95.59

smoothed curve. This may be due to the lack of control over many of the factors involved. It appears, however, to be an exponential curve, a mirror image of the curves relating age to weight and yearly milk production.

It may be concluded from the above data, as well as that presented by Sanders, Gaines and Davidson, that there is a distinct decline in the persistency of secretion during the second and third lactation periods, as compared with the first. As the two most important factors concerned in total yearly production are milk yield during the maximum month and persistency of secretion, and further, that yearly milk secretion increases with age, it is evident that the maximum month's secretion increases rapidly with age. In other words, while the so-called "persistency" character is declining with age up to maturity, the "maximum production" character is not only increasing with age, but

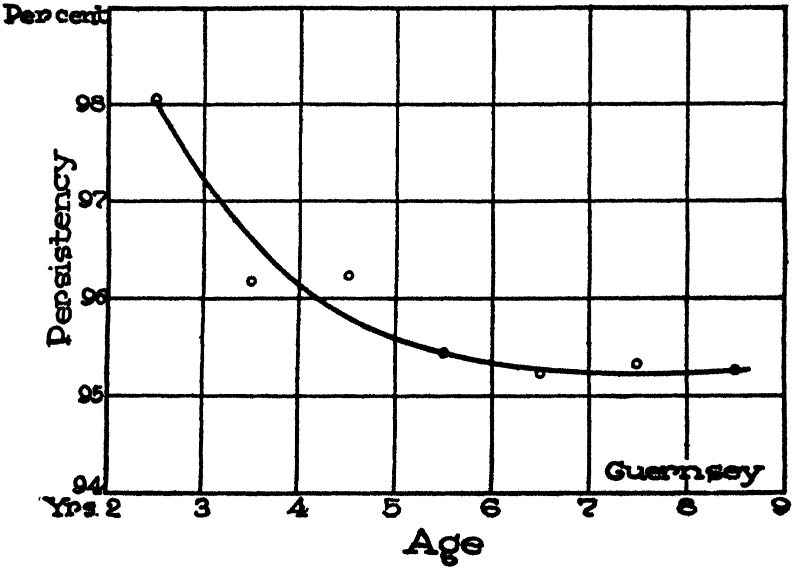

FIG. 1. The change in average persistency with age is indicated. There is a rapid decline which gradually slows up as maturity is reached. The points are the observed values with a smoothed curve of exponential form drawn through them. It will be noted that the curve is a mirror image of the curves relating age to weight and yearly milk production.

at a rate sufficient to overcome the influence of the former on total lactation yield. In advancing a theory to explain these results, it may be desirable to first indicate the source of the stimulation of milk secretion and its relation to the persistency or rate of decline.

THE STIMULATION OF MILK SECRETION

The similarity of the declining curve of milk secretion to the curve of decline of chemical reaction (of the first order type) has been pointed out (5). Milk secretion, even though a very complicated physio-chemical process, might follow a simple chemical law if governed by the slowest process involved. This limiting substance may be of the nature of a chemical catalyst or hormone, which is essential to the chemical transformations taking place in the active secretory tissue of the mammary gland. The consumption of this hormone at a rate in accordance with the exponential decline would furnish the regulating mechanism of milk secretion.

Evidence is gradually being accumulated which indicates the the source of this hormone or at least a hormone which causes active growth and proliferation of secretory cells of the mammary gland, and which eventually initiates a limited amount of secretion. Meyer (6) in a study of albino rats, found that as the time of puberty approaches, the milk ducts begin to proliferate and grow very rapidly, resulting in a dense arborization of ducts. It was observed that these changes in the milk ducts correspond with marked changes in the ovaries and showed large Graafian follicles in the process of development.

Changes in the non-pregnant guinea pig, studied by Loeb and Hesselberg (7), indicate that there is a cyclic change in the mammary gland which corresponds to the cycle in the ovary and uterus. Activity of the gland is at a maximum at the time of heat and ovulation, and gradually falls. At this time, cells in mitotic division are present.

In cattle, Hammond (8) states that after the first heat period the udder enlarges and the ducts grow in length, but it still contains a large proportion of fat. He found that the growth of the milk ducts was due to the functioning of the ovaries and to the formation of corpora lutea.

It appears from the foregoing discussion that puberty and the accompanying changes in the ovaries at that time are responsible for the stimulation of the growth and proliferation of the mammary gland and the stimulation of a very limited secretion.

If pregnancy does not occur with the first ovulation the mammary glands undergo slight retrogressive changes; however, as the next ovulation approaches the mammary gland takes on a new development. Similar changes can be observed in some animals with each succeeding ovulation until pregnancy occurs.

The changes in the mammary gland during pregnancy have been studied in the rabbit by Lane-Claypon and Starling (9) and in the guinea pig, by Loeb and Hesselberg (10). Rapid proliferation of secretory tissue took place during pregnancy after nine days in the rabbit and twenty-four days in the guinea pig. A secretion could be pressed out of the gland during the latter part of the period of gestation.

In cattle, Asdell (11) reports that the amount of liquid that may be milked from the pregnant heifer increases very rapidly and abruptly when the animal is about half way through pregnancy. When a pregnant heifer was milked regularly during her first pregnancy, the yield of milk increased rapidly from the mid period until at calving time as much as 5000 cc. were being produced.

Thus it may be concluded that growth and proliferation of the secretory cells of the mammary gland takes place soon after pregnancy occurs. Coincident with it, or following the growth phase, the secretory phase of activity of the gland is begun. Considerable secretion occurs long before the end of pregnancy.

Asdell concludes that "there seems to be no necessity to postulate the production of an inhibitory hormone from the fetus which defers lactation until the fetus, and consequently the inhibition, is withdrawn by parturition. Such is not in accordance with the facts given in this paper, and indeed appears unnecessary, for while a cell is growing and dividing, surely it cannot secrete. The only inhibition is that produced by the excitement of the cell to grow."

The source of the hormone causing the secretion of milk is much debated. The fetal hormone theory was one of the first proposed. Later the placenta and corpus luteum was suggested as the internal gland of secretion of this hormone. As all of these structures are associated with pregnancy, it is difficult to determine which is responsible.

While experimental data are not available, it is quite possible that the follicular hormone from the ovary isolated by Allen (12) may be responsible not only for the stimulation of the mammary gland to proliferation and secretion from puberty to the first pregnancy, but also during pregnancy. A relatively large amount of active material has been recovered from the placenta which by all tests is similar to the follicular hormone. Allen, Pratt, and Doisy (13) make the following comment on the endocrine function of the placenta:

> It is probably too early to draw general conclusions concerning the endocrine function of the placenta. It is rather difficult, however, to see how such large quantities of active substance could be retained in such a vascular organ as the placenta without passing freely into the maternal circulation. Since it is so well established that the development of the follicles is seriously inhibited during pregnancy, it would seem to us more probable that the human placenta takes over from the ovaries the major part of the function of the secretion of this hormone, thus maintaining the maximum function of the genital tract and initiating growth in the mammary glands during gestation.

If the placenta is the source of the hormone stimulating milk secretion during the gestation period, the source of supply would be cut off at parturition. This being the case, the rate of decline of milk secretion would necessarily be confined to the activity of the secretory cells either individually or collectively, and not to a diminution of the hormone at its source.

To account for the reduced rate of decline during the growth period, i.e., up to seven or eight years, it may be postulated that further development of the mammary gland up to its full mature development may be brought about in any or all of the following ways: (*a*) The reoccurrence of the oestrus cycle, (*b*) another pregnancy, (*c*) a larger blood supply to the mammary gland accompanying growth in weight, (*d*) the increasing activation and perfection of functioning of the secretory cells with use.

GROWTH OF THE MAMMARY GLAND

The first two factors which may cause the rate of decline of milk secretion to be retarded would result from the growth of secretory tissue during the lactation period. With more cells beginning to secrete milk, the yield would be augmented to supplement the natural decline. As the data under consideration consisted of lactations of non-pregnant cows, only the first of these two factors could influence the rate of decline of secretion during the growing period of the cows under consideration. It is not unreasonable to assume that the oestrus cycles which reoccur soon after parturition cause further growth of secretory tissue during the lactation period. It is quite evident that the udder increases in size up to maturity and most rapidly during the second and third year. This growth is due to a stimulus other than that associated with body growth as it takes place only when the ovaries function. Little or no mammary development takes place in spayed heifers.

During the first pregnancy, growth of the mammary gland is very rapid. It is undoubtedly stimulated to further growth during the second pregnancy, but due to other factors accompanying pregnancy any stimulating effect would be masked. There is a difference of opinion as to the cause of the diminution of milk secretion during the latter part of pregnancy. Gaines and Davidson (3) have recently presented the side of those who believe that a hormone of pregnancy inhibits milk secretion and causes the decline observed in the data compiled at this Station (14), and by Eckles (15). Ragsdale, Turner and Brody (14) are of the opinion that the decline in milk flow during gestation is in part at least due to the demands of the growing fetus. The recent data by Asdell (11) strongly supports the conclusion that no inhibitory hormone need be postulated to account for the decline of milk secretion.

Using questionable methods of calculating data on the weight of the fetus, Gaines and Davidson (3) compare the weight of the fetus and the rate of decline of milk yield due to pregnancy. The two curves were not found parallel, and therefore they conclude that "it seems unlikely that the rate of milk secretion is affected at all proportionately to the nutrients required by the fetus." Their assumption that the requirements of the fetus at any moment are proportional to its weight would not necessarily be true as it is well known that growth or milk secretion may be limited by a single deficiency. It might well be that milk secretion would be limited by the needs of the fetus for calcium and phosphorus for the development of the skeleton during the latter part of intra-uterine growth which may bear little relation to its change in weight.

The point which it is our desire to emphasize is that pregnancy unquestionably furnishes the "follicular" hormone that causes further growth and development of the mammary gland during the second and succeeding pregnancies up to the time of maturity. This development, however, is masked by the needs of the fetus which over-balance the increased secretion produced, due to the functioning of the new cells present.

ACTIVITY OF THE MAMMARY GLAND

The other factors which may influence the rate of decline or the "persistency" of milk secretion as the animal reaches maturity, would rest with the activity of the secretory cells present. If this would change for any reason, the persistency would follow a similar but inverse course. A close relation has been found between weight and yearly fat production as the dairy cow reaches maturity (4). There is a quite similar increase in weight and fat production up to seven or eight years in the Jersey cow. For each 100 pounds increase in live weight above 472 pounds, there is a corresponding 100-pound increase in yearly fat production. Within a single age class, however, there appeared to be an increase of approximately 20 pounds of fat for each 100-pound increase in live weight. This was taken to indicate that the change in fat production with age is probably due to two separate factors; an increase in body weight contributing about 20 per cent to the total increased fat yield, while 80 per cent of increased fat yield is due to other factors accompanying increased maturity.

During the first lactation period of a heifer and to a lesser extent with older growing animals, the increase in weight and corresponding increase in the volume of blood will supply the mammary gland with a larger amount of blood. This increased blood supply is thought to be in part responsible for retarding or masking the natural (hereditary) rate of decline of secretion.

With the removal of milk either before or after parturition, the cells must gradually become adjusted to the changed conditions. The increasing activation and perfection of the functioning of the secretory cells to secrete milk up to their full capacity may also change with maturity of the animal. This change would also retard the natural decline in the rate of secretion.

SUMMARY

1. Using a method previously described for the quantitative determination of persistency of milk and fat secretion, a study was made of the effect of age upon this character.

2. Data were presented indicating that there is a decline in the persistency of secretion during the lactation period as the dairy cow reaches maturity.

3. A summary is made of the literature indicating the nature of the stimulation of mammary growth and secretion and the relation to the problem of the influence of age was indicated.

4. The theory is advanced that the normal or hereditary rate of decline of milk secretion during the early lactation periods is retarded due to further division of cells which become functional through the stimulation of the "follicular" hormone, and to the increased activity of the cells due to an increased blood supply and the perfection of the functioning of the cells.

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A NEW METHOD OF MANUFACTURING CREAM CHEESE OF THE NEUFCHATEL TYPE*

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Cream cheese is a soft, fresh cheese with a rich, mild acid flavor; a smooth, buttery texture; and a high percentage of milk fat. Strictly speaking, cheddar or American cheese, which is a hard-ripened cheese made from milk, should not be called cream cheese, even though it is often referred to as cream cheese and some state laws recognize it as full cream cheese when it is made from normal milk. Cream cheese is normally made by the neufchatel process from cream or from milk to which cream has been added.

The extent of the manufacture of cream cheese has increased greatly in recent years. Cream and neufchatel cheese constitute one of the leading varieties of cheese manufactured in New York State. The last available statistics for New York State[1] show that in 1924 there was sold to dairy plants for manufacture into cheese the following number of pounds of milk:

KIND OF CHEESE	POUNDS OF MILK
American or cheddar	43,500,000
Cottage, pot and bakers	19,700,000
Cream and neufchatel	9,900,000
Limburger	4,200,000
All other kinds	7,900,000

The percentage of fat in cream cheese varies from 38.6 to 48.0, according to Matheson, Thom, and Currie.[2] The same authors give the moisture content as varying from 30 to 48 per cent.

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[1] Statistics relative to the dairy industry in New York State, 1924, Bul. 180, Department of Farms and Markets, 1925.

[2] Matheson, K. J., Thom, Charles, and Currie, J. N. Cheeses of the neufchatel group. Conn. Agr. Exp. Sta. (Storrs), Bul. 78, 1914.

The richness of cream cheese should be evident in the characteristics of the cheese when compared with neufchatel or cottage cheese. Cream cheese should have a mild acid, distinctly creamy flavor. The acid and salt content should be lower than in cottage cheese. The texture is usually described as very smooth and buttery. It should spread almost as freely as butter. The body should be more rigid than cottage cheese so that cream cheese can be sold in boxes and packages. It should not be crumbly or too sticky and should pack very solidly. These properties make it possible to cut cream cheese loaves into slices which do not break and which have a solid surface.

The retail price of cream cheese is usually higher than the price of butter and several times greater than the price of cottage cheese. The consumer buys it in small quantities, one-half pound or less and appears to consider it a delicacy. While much of this cheese is consumed as any ordinary cheese would be, it is especially well considered as a spread for crackers in place of butter, as a filling for sandwiches, and in salads. This cheese is often mixed with ground pimento, nuts, olives, mixed sweet pickles, and salad dressings to give variety, especially when used in sandwiches.

THE NEUFCHATEL PROCESS

Investigations upon the manufacture of cream cheese are somewhat limited in number, although the methods of manufacture often appear in the literature. Matheson, Thom, and Currie give the details of the process and stress certain points of special importance. The milk must be low in acid and of excellent quality. A low quantity of good starter and rennet should set the milk at 70° to 75°F. in twelve to eighteen hours. The curd should be drained in sacks at a temperature of 50°F. and in a humid atmosphere. Pressure is essential to expel the last traces of free whey. The finished cream cheese should contain 38 to 43 per cent water, 43 to 48 per cent fat, and 0.5 to 1.25 per cent of salt.

Marquardt[3] improved the process in several respects. Pasteurization and commercial starters improved the flavor and keeping qualities of the cheese. Homogenization of the milk to which cream had been added reduced the fat lost in the whey and permitted the use of richer cream without excessive fat losses.

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[3] **The investigations by J. C. Marquardt, New York Agricultural Experiment Station, have not yet been published.**

Certain difficulties in the present manufacturing process are occasionally encountered in commercial practice. Lack of a proper control of temperature conditions during setting and draining, combined with variations in rennet action and acid development, may result in a somewhat granular, crumbly cream cheese. Draining of the whey from the curd in the bags is usually slow and the acid development may result in a sour or yeasty cheese. Proper drainage of sufficient amounts of whey may take too long a time so that the manufacturer may pack the cheese too wet. A cheese with a high moisture content keeps poorly.

PLANS OF THIS INVESTIGATION

The results of studies of homogenization and the fat clumping in milk and cream at this station made it reasonable to suppose that through homogenization of cream its viscosity or plasticity might be increased to such an extent that a body and texture suitable for cream cheese would be produced. Consequently, cream was standardized to compare in composition with cream cheese. The cream was pasteurized and homogenized. The result of the first trial was encouraging enough to warrant a rather detailed study of the method, especially considering the evident advantages of the method if it proved successful. The method is also of scientific interest and might serve as a basis for other investigations of similar character. Consideration was given to the effect of varying percentages of fat and total solids, of varying temperatures and pressures of homogenization, etc.

Unless otherwise stated in the manuscript the Manton-Gaulin homogenizer, Type 60, with the single stage valve, was used in the manufacture of the cream cheese.

THE METHOD

The new method can be presented to best advantage by giving, first, the final method in detail as adopted, and later, by giving the effect of variations from the standard method.

Sweet cream of good flavor containing 40 to 45 per cent of fat was the basis for the cheese. To this cream 5 per cent of soluble dry skimmilk of good flavor was added. Either 1 per cent of high grade powdered pure food gelatin or 0.5 per cent of powdered agar free from objectionable flavor and odor was stirred into the cold cream.

This mixture was then pasteurized at 145°F. for thirty minutes if gelatin was used, and at 180° to 185°F. for ten minutes if agar was used. After pasteurization the mixture was cooled to 110°F. Common salt to the extent of 0.75 per cent, and 0.5 to 1.0 per cent of good commercial starter were then added. The mixture was passed through a coarse strainer into the homogenizer. The homogenization pressure of 3500 to 4000 pounds per square inch gave best results. The cream as it left the homogenizer had the consistency of soft butter or was slightly stiffer than ice cream as it comes from the freezer. The warm cream mixture was usually raised 5 to 10 degrees in temperature during the homogenization process.

The cream mixture was immediately placed in the final container and allowed to stand in a refrigerator at 32° to 40°F. for one or two hours until the temperature of the cheese was reduced to 70°F. The cheese was held at 70°F. for ten to fifteen hours or until it had developed sufficient acid to have a mild acid flavor. The product was then considered to be cream cheese. It was stored at 32° to 40°F. and was ready for immediate consumption. At this temperature cheese was held for two to five weeks without undergoing any serious deterioration in flavor.

Cream cheese made by this process was always smooth and buttery in texture without any traces of crumbliness. Its body was firm. The cheese possessed excellent spreading qualities and could be sliced as well or better than cream cheese made by the old process. It looked and tasted richer then the ordinary cream cheese, even though it did not contain a higher percentage of fat.

INFLUENCE OF VARYING PERCENTAGES OF FAT

Experimental cream cheese were prepared containing 30, 35, 40, 45, and 50 per cent of milk fat. Increased percentages of fat caused increased firmness of body and decreased the quantity of whey that would drain from the cheese under adverse conditions.

Cheese containing 30 per cent fat was quite unsatisfactory, due to its soft sticky condition and leakage of whey when warmed to 70°F. or above. Under ideal conditions 35 per cent of fat was satisfactory, but this quantity of fat would not be sufficient for commercial conditions. The most satisfactory cheese was made with 40 to 42 per cent of fat and unquestionably the flavor was finest with this richness. When this cheese was stored above 70°F. some whey drained from it. Although this small amount of whey drainage is objectionable, it must not be forgotten that cream cheese is perishable and should always be kept in a cold place. If the cheese is stored above 70°F., it will spoil quickly irrespective of whey drainage. The body of the cheese with 40 per cent of fat was sufficiently firm and free from stickiness to permit the proper slicing of cheese loaves. The flavor was rich, creamy, and mildly acid. The texture was very smooth, buttery, free from crumbliness, and the cheese spread easily.

When the percentage of fat was increased to 45 per cent two slight alterations in the cheese were made. The flavor was somewhat richer and a few people who ate the cheese plain without other foods said that it was a little too rich. Cheese with this percentage of fat did not leak whey at any temperature up to 85°F. When 50 per cent of fat was used the cheese was noticeably too rich in flavor for many people who ate it.

A limited number of fat globule measurements were made to show the effect of homogenizing the cream mixture upon the size and distribution of the fat globules. One cubic centimeter of cream was diluted with 99 cc. of distilled water and examined in hanging drop preparations. The fat globules in the cream after pasteurization and prior to homogenization were 4.2 microns in diameter, and in 100 fields examined all existed as individuals. After homogenization the size of individual fat gobules was determined from those existing singly because of the difficulty of measuring globules present in clusters. The size of the clusters or aggregates of fat globules and the ratio of individuals to cluster were determined. A total of 128 fat globules had an average diameter of 2.04 microns. In the same microscopic fields there existed 219 clusters with an average size of 11.9 by 8.2 microns. It was evident that most of the fat globules in the homogenized cream existed in large clusters of globules about one-half their original size.

The aggregation of the fat globules formed a network in which the milk serum was held. The body and texture of the cheese was probably due to this changed condition of the fat, a change which might be considered in colloidal chemistry as a change of the fat from the dispersed to the continuous phase.

INFLUENCE OF VARYING PERCENTAGES OF MILK SOLIDS NOT FAT

An attempt was made early in this work to make a firmer cheese which would not leak whey by increasing the percentage of milk solids not fat through the use of dry skimmilk. Increased percentages of milk solids not fat reduced the rich fat flavor to a considerable degree and improved the body by making it slightly firmer and less sticky. It did not have any marked effect upon whey drainage which was so greatly altered by the percentage of fat.

The percentage of milk solids not fat was held low for two reasons. If 10 per cent or more of dry skimmilk was used, the finished cheese could develop too high a percentage of acid at too warm storage temperatures. Furthermore, when exposed to drying conditions the milk sugar crystallized out and formed a grainy coating on the cheese. The addition of 5 per cent of skimmilk solids is desirable to decrease the buttery taste of the cheese, to increase slightly the firmness of the cheese, and to give the cheese better slicing properties. It probably would have been desirable to have omitted the additional milk sugar, but no feasible method was apparent by which the skimmilk solids could be obtained without the sugar.

INFLUENCE OF VARYING CONDITIONS OF HOMOGENIZATION

Greater homogenization pressures were essential in the production of cream cheese by the new method than are commonly employed in the manufacture of other dairy products. As a general proposition it was found that pressures varying from 3500 to 4000 pounds per square inch produced a firmer bodied cheese with less stickiness and less drainage of whey than did lower pressures. When the fat content was high, 45 to 50 per cent, 500 pounds less pressure was sufficient and 4000 pounds pressure produced a cheese which was somewhat too rich in taste and resembled, to some extent, cream almost ready to form butter in the churn.

Any temperature of the cream above 105°F. was suitable for homogenization so far as the body and texture of the resulting cheese were concerned, but it is obvious that starter could not be safely added to cream homogenized at a temperature much in excess of 110°F. because the temperature was increased 5° to 10°F. by homogenization. In several tests the cream was homogenized at 145°F., cooled to 70°F., and starter added. The salt was sometimes added with the starter and also after the cheese had ripened to the proper degree. No special advantage could be determined in this method of adding salt and starter after homogenization, and it had the disadvantages of making extra work and the excessive mixing of the cheese tended to make it sticky.

Cream cheese was also prepared from cream which had been pasteurized, cooled, and ripened with commercial starter at 70°F. The cream was then pasteurized again, salt was added, and the whole mixture with the proper percentage of acid was homogenized. By this method it was hoped that the acid development would be checked even when the finished cheese was stored at too warm a temperature. The method proved unsatisfactory because the homogenization of sour cream produced a cheese with a soft sticky body from which whey drained freely.

Commercial rennet was tried by adding it when the starter was added in the recommended process. The rennet gave the homogenized product a more buttery appearance and taste, and whey drained much more freely from the cheese. When added after homogenization the rennet caused increased drainage of whey. For these reasons the use of rennet in the manufacture of this cheese was not desirable.

The cream was homogenized at colder temperatures with poor results. The fat was not properly homogenized, the cream appeared to be partially churned, and the body of the cheese was softer than usual. Whey drained from the cheese quite freely. Measurements of 100 fat globules in cream homogenized at 90°F. showed them to be 3.5 microns in diameter or only slightly smaller than those in normal cream. Only 46 clumps measuring 11.6 by 9.7 microns were found per 100 individual fat globules, showing a great reduction in the aggregation of the fat globules.

RIPENING THE CHEESE

The quantity of starter required to develop the proper acidity was dependent upon many conditions, but 0.5 to 1.0 per cent usually proved successful.

The percentage of salt added to the cheese had an effect not only upon the rate of acid development, but also upon the kind of flavor produced. When the percentage of salt was held below 1.0, the salt did not seriously interfere with the growth of the starter organisms.

Most starters are past the period of maximum activity when added to dairy products for ripening purposes, so that a period of lag was always encountered. In cream cheese, due to the added effect of heating the starter from 110° to 120°F., considerable time elapsed before acid development commenced. For these reasons speed in cooling the homogenized cream was not very essential. In cold weather good results were secured by placing the warm cheese as it came from the homogenizer in the room for ripening at 70°F. without previous cooling. In the summer this procedure could not be followed because the cheese became sour without developing desirable flavors. For this reason it was deemed advisable always to cool the cheese to 70°F. by placing it in a refrigerator previous to ripening.

THE USE OF AGAR AND GELATIN IN CREAM CHEESE

Agar and gelatin were tried in the cream cheese for the purpose of holding the whey so that none would drain out even though the cheese were stored too warm. The quantity of agar was varied from 0.2 to 1.0 per cent and the quantity of gelatin from 0.5 to 2.0 per cent. Both the agar and gelatin were dissolved in hot water and stirred into the cream, and they were dissolved by mixing the dry powders directly into the cream.

The water required to dissolve the agar or gelatin was a distinct disadvantage because it increased the percentage of water in the cheese. Hence, after several trials, this method was discontinued and the dry gelatin or agar was added directly to the cream and dissolved by pasteurization. The gelatin presented no problem in this respect because it dissolved readily at the ordinary pasteurization temperature of 145°F. Tests[4] were made to determine the temperature and time required to dissolve powdered agar in water. It was learned that powdered agar began to dissolve at approximately 170°F. and a temperature of 180°F. for ten minutes seemed to dissolve the agar as well as did the boiling temperature. For this reason the powdered agar was dissolved in the cream by pasteurization at 180° to 185°F. for ten minutes.

Several tests with varying percentages of gelatin and agar showed that 1.0 per cent of gelatin or 0.5 per cent of agar gave best results. Since the cream cheese contained 50 per cent or less of water, the actual concentration of gelatin in water was 2.0 per cent and of agar 1.0 per cent. Both of these percentages were sufficient to form a jelly at room temperature. The presence of these gelatinizing substances produced a cheese with improved slicing qualities and a slightly firmer body. They also reduced the tendency of cream cheese to exude whey at warm temperatures, but neither weak agar nor gelatin jellies in the cream cheese could hold the whey so that some of it would not drain out of the cheese if the cheese were improperly made in other respects. The presence of agar or gelatin in the cheese was distinctly advantageous.

The powdered agar used for cream cheese making was of excellent quality and practically free from flavor and odor. Powdered pure food gelatin of high quality, such as that used in ice cream, proved to be an excellent grade for this purpose. The use of agar had the disadvantage of requiring a high temperature to dissolve it, and its use had the advantage of making a firmer bodied cheese of improved slicing qualities. The use of agar is especially recommended.

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[4] These tests were made by J. C. Marquardt of this Station.

ADVANTAGES AND DISADVANTAGES OF THE NEW METHOD

The new method of manufacturing cream cheese had several distinct advantages over the old neufchatel process.

When the milk for cheese making was received it was immediately reduced to the same approximate bulk as the finished cheese by separating into rich cream. This reduced the volume of cream which needed to be handled. No bags for drainage were needed. The acid development could be checked at any time by cooling the cheese because the moisture content was standardized before the cheese was made. This permitted better control of the extent of acid development and prevented spoilage of cheese by too much acid in a slow draining curd.

The cream cheese could be made more uniform in body and texture by the new process. The body and texture were determined largely by the composition of the cheese, the sweetness of the cream, and homogenization temperatures and pressures. These factors were readily controlled and uniform cheese was made. The labor needed to make cream cheese by the new process was considerably reduced.

Cream cheese of more desirable characteristics was made by the new process. It was smoother and more buttery, tasted richer, spread better, and sliced as well or better than cheese made by the neufchatel method. Its flavor from the standpoint of acid development and bacterial growth could be controlled to better advantage and the cheese was finished and in the refrigerator twelve to twenty-four hours sooner when made by the new method. The cheese could be packed more solidly in loaves or packages, and probably because of the exclusion of air, it did not mold as readily under improper storage conditions. The cheese was never crumbly.

It seldom occurs that a new method is proposed which in all respects is advantageous to use. Cheese made by the new method had too high a milk sugar content which permitted the excessive development of acid under warm storage conditions. This disadvantage was offset to some extent by a reduced mold growth. Cheese made by the new method was considered too rich and buttery tasting to suit a few people, but it should be stated that this cheese was consumed in rather large quantities before this characteristic was discovered. It was essential that rich tasting cheese be made by this process, because a low percentage of fat was inducive to leakage of whey. Cheese of a given percentage of fat tasted richer when made by the new process.

The cheese made by the new process was more pasty than regular cream cheese. However, it sliced just as well as any neufchatel cream cheese made experimentally or purchased in the local market. All cream cheese is too sticky to cut with a knife. It should be sliced with a wire or string after the tin foil surrounding the cheese has been cut with a knife.

MILK YIELD IN RELATION TO RECURRENCE OF CONCEPTION*

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INTRODUCTION

Lactation is known to affect the reproductive cycle in man and some of the lower animals. Hammond (1) has shown experimentally in the rabbit that when lactation proceeds at a high level pregnancy is apparently inhibited. It seems to be a prevalent opinion that the fitting and management of cows on official test to secure as high a level of milk production as possible has a tendency to interfere with the occurrence of conception. The relation of the rate of milk secretion to the recurrence of pregnancy is not only of physiological interest, but in the case of the cow it becomes also of much economic interest.

It is well known that the economy of milk production in the cow is very closely related to the amount of milk produced, expressed as an average per year over the life of the cow. The factors contributing to the average yield per year include three major physiological elements: (*a*) frequency of calving; (*b*) initial or maximum rate of milk yield following calving; (*c*) persistency of lactation or the rate of decrease in the rate of yield with advance in lactation. These three elements may be regarded as given in order of their importance, inasmuch as the bearing of young is ordinarily essential to the initiation of milk secretion, and inasmuch as initial rate of yield is a much more powerful factor determining yearly yield than is persistency of lactation.

The common practice of dairymen is to breed their cows to freshen about once a year and this practice is essential to efficient milk production regardless of the value of the calf itself. Frequent calving is necessary to renew the stimulus to milk secretion and essential to maintain the mammary gland at the highest

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average rate of secretory activity. This may be demonstrated from theoretical premises. It is well known that the average lactation curve is of a descending exponential type [cf. (2), (3), (4)] terminated more or less abruptly by advanced pregnancy through natural and artificial agencies [cf. (5), fig. 9]. The theory of the case is set out in figure 1.

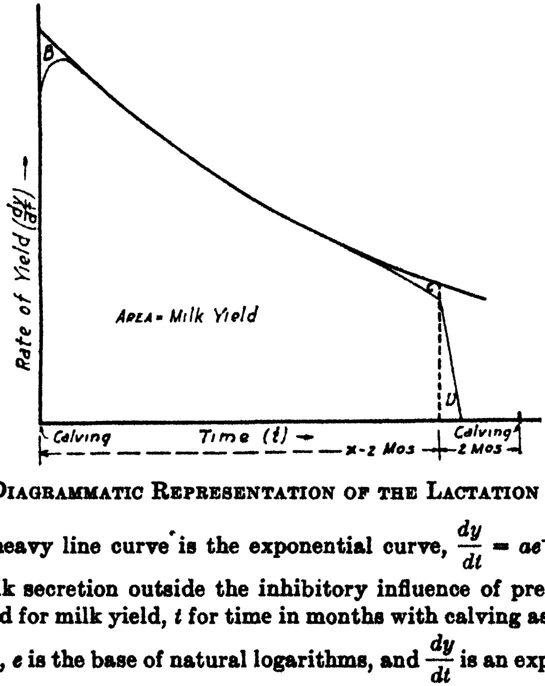

FIG. 1. DIAGRAMMATIC REPRESENTATION OF THE LACTATION CURVE

The upper heavy line curve is the exponential curve, $\frac{dy}{dt} = ae^{-kt}$, typical of the rate of milk secretion outside the inhibitory influence of pregnancy. The symbol y is used for milk yield, t for time in months with calving as origin, a and k are constants, e is the base of natural logarithms, and $\frac{dy}{dt}$ is an expression of the calculus which in this case, means the instantaneous rate of milk yield per month. This equation is admirably adapted to describe the lactation curve because of its close agreement with the observed milk yields and because of the properties of its constants. The constant a represents the initial rate of yield per month; k represents the rate of decrease per month in the rate of yield; and the area under the curve, that is the total amount of milk yielded up to any time (t) is given as a function of the time by the expression $\frac{a}{k}(1 - e^{-kt})$.

The lower or coincident lighter line curve represents the average realized lactation curve. The full rate of milk secretion required by the simple exponential curve above is not realised until a few days after calving [cf. (6) and (7), fig. 4]. This results in the small area or deficiency in yield marked B in the diagram. It is possibly due to a residual inhibition from the preceding pregnancy. Some time after calving the cow again conceives and at a somewhat advanced stage of preg-

The average yield per year for the period from one calving to the next, denoting this period in months by x, becomes:

$$12a\left(\frac{1 - e^{-k(x-2)}}{kx}\right)$$

Ordinarily, k = about 0.1 and assigning it this value and considering a constant, we find that the value of the expression decreases as x increases beyond 10 (about the lowest possible value of x). Or, to leave the matter in mathematical form the expression reaches a maximum when $kx + 1 = e^{k(x-2)}$. This equality with $k = 0.1$ is attained when $x = 7.72$. Even if $k = 0.05$, as is possible under liberal feeding, the maximum is reached at $x = 10.32$. Hence, the highest average yearly yield is obtained by the most frequent breeding, assuming that a remains constant. Ordinarily, of course, a increases with each succeeding lactation up to an age of eight to nine years, so that the desirability of frequent reproduction is further emphasized.

Furthermore, a and k are closely correlated as are also a and y, while k and y are only slightly correlated.[1] It is clear that

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nancy there is again an appreciable reduction from the full rate of yield required by the upper curve. This results in the small area or deficiency in yield marked C in the diagram. It is probably due to an inhibition of milk secretion associated with the current pregnancy. Finally, at two months before term (broken vertical line in the diagram) the cow is dried off, a yield represented by the area D being produced during the process. The cow is dried off in anticipation of the following lactation and a dry period of six or seven weeks is considered sufficient [cf. (5), fig. 16].

An expression for the area under the lower lighter line curve representing the realized milk yield might be derived but it would be rather complicated. As a usable approximation we may assume that D = B + C in the diagram and estimate the lactation yield from the simple upper curve, since B, C and D are small as compared to the total area. If we denote the time in months from one calving to the next by x then the lactation yield becomes $\frac{a}{k}[1 - e^{-k(x-2)}]$. From this expression we may readily generalize as to the effect of variation in length of the calving interval on average yield.

[1] Guernsey records treated on an energy basis, and dealing with yield (Y) for 365 days of the lactation show $r_{ak} = 0.535$, $r_{aY} = 0.672$, $r_{kY} = -0.226$ (paper in press). It was first shown by Gavin (8) that lactation milk yield is closely correlated with the maximum day's yield, $r = 0.839 \pm 0.006$.

high yearly yield is to be attained only from high initial or maximum rate of yield and high initial rate of yield carries with it a rapid rate of decrease in rate of yield with advance in lactation. The larger k the more rapidly the expression of the preceding paragraph decreases as x increases. The necessity of frequent reproduction is therefore still further emphasized.

The foregoing preamble may serve to indicate the significance of the relation of the rate of milk secretion to the occurrence of pregnancy, from the standpoint of economical milk production. If high initial rate of milk secretion interferes with conception in the cow as Hammond has shown it does in the rabbit, then the advantage that is attained by the capacity to high yield at the flush of lactation will be lost, more or less, by the failure to conceive again promptly. The published records of the American Guernsey Cattle Club afford data of value bearing on this point, and certain of these records are presented in the present paper as bearing on the relation of the rate of milk secretion to service period.

SOURCE AND TREATMENT OF DATA

The records are taken from Volumes 33, 34 and No. 1 of 35 of the Guernsey Herd Register, and include all records in the volumes specified in which not more than sixty days elapsed from calving to the beginning of the first full calendar month of the record. The great majority of the records start within a few days after calving. The records of production are published by calendar months and include also data as to time of calving and the effective service following. It is, therefore, possible to correlate the service period with the approximate initial or maximum rate of milk secretion, taking this latter to be the milk yield for the first recorded full calendar month.[2] The records number 4893 of which 222 show no account of effective breeding up to 380 days after calving.

If the rate of milk secretion affects the occurrence of pregnancy it may be presumed that the effect will vary with the age of the cow, that is, a rate of yield of 1200 pounds per month by a two-year old would be presumed to interfere more seriously with pregnancy than would the same rate of yield in a mature cow. The records have, therefore, been classified according to the age of the cow. For this purpose the classification used in the published records has been followed, viz.: Class A, five years and over; B, four and one-half to five; C, four to four and one-half; D, three and one-half to four; E, three to three and one-half; F, two and one-half to three; G, less than two and one-half years. Class A includes cows up to sixteen years or more of age. Class G includes a small proportion (10 per cent) of cows under two years, with rarely any (0.4 per cent) below one and one-half years. Age is reckoned according to the date at which the record starts.

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[2] As a saving in the labor of computation the calendar month milk yields are used directly, ignoring the variation in length of the months and in the fat percentage of the milk.

RESULTS

The means, standard deviations and coefficients of variability for rate of yield and service period are given in table 1, also the coefficients of correlation between these two variables. Cows not bred at 380 days after calving are excluded in table 1. It is apparent from the constants that while the mean rate of yield changes markedly with age, there is no material or consistent change in the mean service period with age. Judged by the coefficient of variability, service period is a highly variable feature of these records.

The coefficient of correlation between rate of yield and service period at the various ages shows a small positive correlation except at the three to three and one-half year old class. The correlation for all ages, $r = 0.039 \pm 0.010$, may be regarded as significant from a technical statistical standpoint, but it is evident for all practical purposes that the first full month's milk yield and service period are independent.

Table 2 gives the mean service period for various age classes at different levels of milk yield. There is given also, in the last column, the number of cows not bred at 380 days after calving, expressed as a percentage of the total number in the respective

TABLE 1

Statistical constants of rate of milk secretion and service period

Guernsey records

AGE CLASS	NUMBER OF RECORDS	MILK YIELD FOR FIRST FULL CALENDAR MONTH			SERVICE PERIOD			COEFFICIENT OF CORRELATION
		Mean	Standard deviation	Coefficient of variability	Mean	Standard deviation	Coefficient of variability	
years		*pounds*	*pounds*		*days*	*days*		
Under 2½	1,120	875	177	20.23	180	80	44.62	0.041 ± 0.020
2½ to 3	523	943	186	19.69	173	80	46.26	0.046 ± 0.029
3 to 3½	434	1,025	191	18.63	162	84	51.61	−0.064 ± 0.032
3½ to 4	416	1,076	207	19.26	169	82	48.63	0.005 ± 0.033
4 to 4½	362	1,146	216	18.86	171	81	47.47	0.033 ± 0.035
4½ to 5	311	1,168	231	19.80	165	81	48.77	0.073 ± 0.038
5 and over	1,505	1,234	231	18.72	177	81	46.13	0.046 ± 0.017
All ages	4,671	1,071	253	23.61	174	81	46.85	0.039 ± 0.010

TABLE 2

Mean service period at various rates of milk secretion and ages

Guernsey records

MILK YIELD FOR FIRST FULL CALENDAR MONTH, CLASS MID-POINTS	AGE, YEARS								COWS NOT BRED AT 380 DAYS
	Under 2½	2½ to 3	3 to 3½	3½ to 4	4 to 4½	4½ to 5	5 and over	All ages	
	Mean service period								
pounds	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*per cent*
450	140							140	0
550	185	212		230	10			181	0
650	168	171	179	203	160	90	140	168	4.2
750	184	161	173	158	148	159	178	175	5.4
850	177	175	172	155	162	178	182	174	3.3
950	177	177	162	161	170	151	175	170	3.4
1,050	186	176	156	188	171	156	171	178	5.1
1,150	195	180	158	172	162	153	167	169	5.4
1,250	181	190	147	139	172	185	169	168	4.8
1,350	186	144	182	187	183	189	184	183	2.8
1,450		187	158	180	174	148	171	170	5.5
1,550	170	226	157	114	153	155	182	174	6.4
1,650			110	220		190	194	190	7.8
1,750			110	170	230	194	189	189	9.5
1,850				150	135	170	240	197	12.5
1,950							216	216	12.5
2,050									0
2,150							200	200	0

milk yield class. The proportion of farrow cows does not change with the rate of milk yield except in the last few (highest yield) classes and here the frequencies are too small to make the results

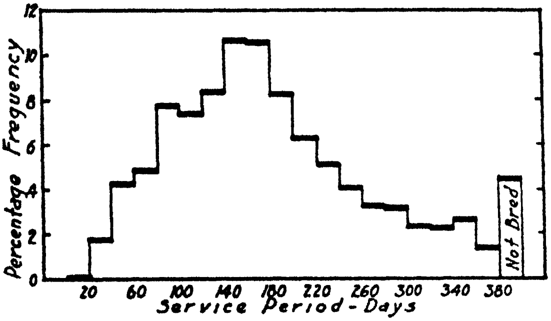

FIG. 2. SHOWING DISTRIBUTION OF 4893 GUERNSEY A. R. RECORDS WITH RESPECT TO SERVICE PERIOD, THAT IS, TIME FROM CALVING TO EFFECTIVE SERVICE

The column at the right indicates cows not in calf at 380 days after calving

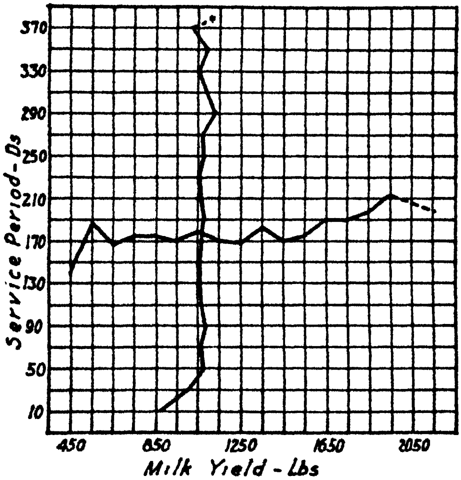

FIG. 3. SHOWING THE OBSERVED REGRESSION LINES DERIVED FROM THE CORRELATION SURFACE FOR THE VARIABLES, FIRST FULL CALENDAR MONTH MILK YIELD AND SERVICE PERIOD

The * shows the mean milk yield, 1111 pounds for cows not bred at 380 days.

of much significance. It may be inferred, therefore, that the absence of these farrow cows in the data of table 1 does not disturb the significance of the correlations there given.

The service period frequency distribution for all ages combined is shown graphically in figure 2, expressed on a percentage basis to the total number of records (4893) as a base. The column at the right represents the 222 cows unbred at 380 days (4.54 per cent) and the area of the histogram represents 95.46 per cent.

DISCUSSION

The present records do not give any information as to infertile services. There is, no doubt, a tendency on the part of the breeders to delay service in the case of cows on official test in order to secure a higher 365-day record. The mean service period, 174 days, is excessively long from the standpoint of economical milk production, and represents one of the consequences of the endeavor of the system of Advanced Registry to obtain a maximum 365-day record. At least there is no apparent reason to suppose that there is any physiological necessity for the unduly long service period of advanced registry practice.

It would seem that there might be a more or less well defined tendency to delay breeding more frequently and for a longer time with cows that start their record at a high level as compared with those starting at a lower level. Such a tendency would lead to a positive correlation between rate of yield and service period. The very low correlation ($r = 0.039$) found may be due to this tendency, but in any case, it seems safe to conclude from the evidence of the present records, that high initial or maximum rate of milk secretion is not antagonistic to the recurrence of conception. The most promising possibility of increasing the average yearly yield, and thereby the efficiency of the cow, seems to lie in increasing the rate of milk secretion at the flush of lactation. It is fortunate if improvement in this direction is not to be neutralized in any wise by a concomitant delay in conception.

SUMMARY

The first full calendar month milk yield and the service period (calving to conception) of 4671 Guernsey A. R. records show a coefficient of correlation of 0.039 ± 0.010. There is no evidence that high rate of milk secretion interferes with the recurrence of conception in these records. The mean service period is 174 days, standard deviation 81 days, coefficient of variability 47. From generalized premises the service period is far too long for the most economical life time production. The records for the same period show no record of breeding up to 380 days for 222 cows (= 4.54 per cent of 4893).

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THE FORMATION OF ACID IN MILK BY HEATING*

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The changes in the hydrogen ion concentration and acidity caused by heating skimmilk at the boiling temperature and at 95° in open flasks have been presented and discussed briefly in an earlier article (1). The rather small amount of literature on the subject was reviewed, and our experimental methods were described in detail. This article contains similar experimental data obtained at higher temperatures and other data bearing on the buffer action of heated milk and on the source and nature of the acid formed.

In carrying out the heating experiments above the boiling point of milk, it was necessary to use sealed containers. The ordinary 6 ounce evaporated milk cans sealed by a crimping machine were employed. Thus there was no possibility of contaminating the samples with the acid of soldering flux. The cans were heated in a steam jacketed kettle containing glycerine, in which the cans were completely submerged. The steam pressure in the jacket was adjusted by a reducing valve to give the desired constant temperature (±0.5°) as measured in the glycerine. All determinations made at the same time interval from the beginning of the heating were carried out on the contents of a single can. With the exception of the acidities on the run at boiling temperature under reflux, in which a Hildebrand electrode was used for the titration, all acidities recorded in this paper were determined by adding to a sample a volume of 0.1 N alkali estimated from previous experience to be sufficient to bring the sample to pH 8.3. The hydrogen ion concentration of the mixture was then determined by means of the Clark electrode. The same procedure was

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repeated with a different volume of alkali until values were obtained between which the exact volume of alkali necessary to give a pH of 8.3 might properly be interpolated. This method avoids diffusion errors and the slow and uncertain approach to equilibrium which was experienced in using the Hildebrand electrode in the earlier titrations.

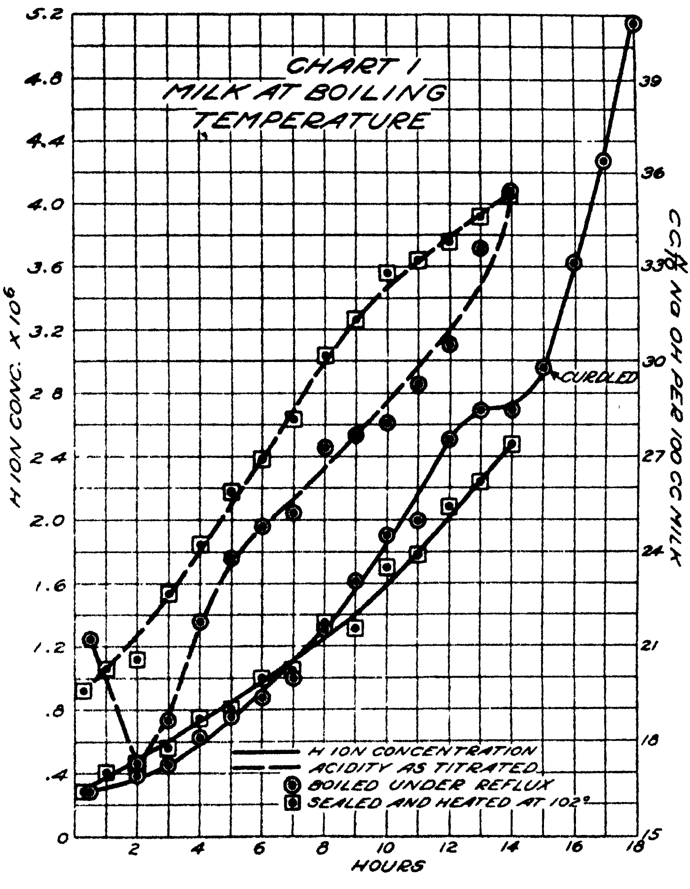

Fig. 1. Effect of Boiling Temperature on Titratable Acidity and Hydrogen Ion Concentration of Milk Held under Reflux and in Sealed Cans

Obviously the closed containers would retain carbon dioxide and any other volatile constituent of the milk whether present originally or formed during the heating. In order to determine to what extent previous data obtained at lower temperatures in open containers could be compared with data at high temperatures in closed containers, a run was made at 102° in sealed cans for direct comparison with a run made at approximately the same temperature (boiling) in a flask under a reflux condenser. The samples of milk were from the same herd but not from the same milking. For these two experiments, hydrogen ion concentration and acidity are plotted against time of heating in figure 1.

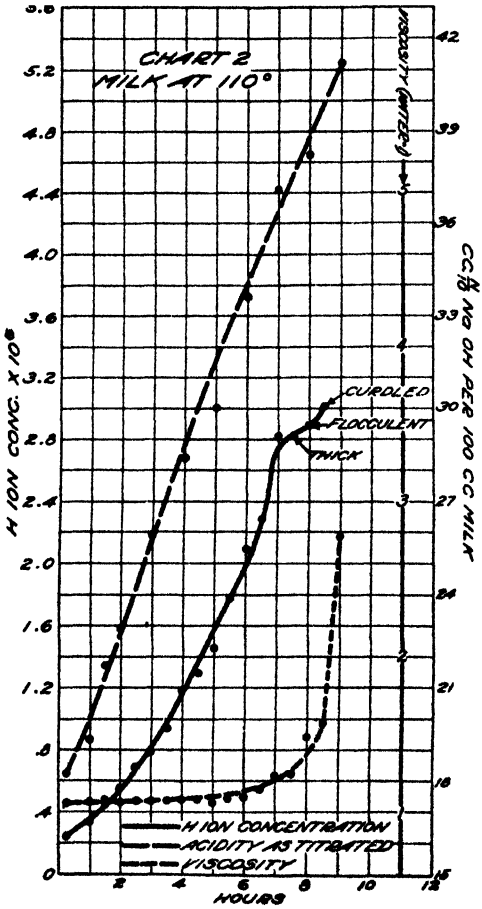

FIG. 2. EFFECT OF HEATING AT 110° ON TITRATABLE ACIDITY, HYDROGEN ION CONCENTRATION AND VISCOSITY OF MILK HELD IN SEALED CANS

It will be noted that the slopes of the curves of each function are practically the same for both samples. The slope of the hydrogen ion curve of the sealed sample is slightly lower than that of the other, probably due to a great extent to the buffer action of the carbonate radical, but possibly due in a measure to a different buffer action of the original milks. The acidity levels

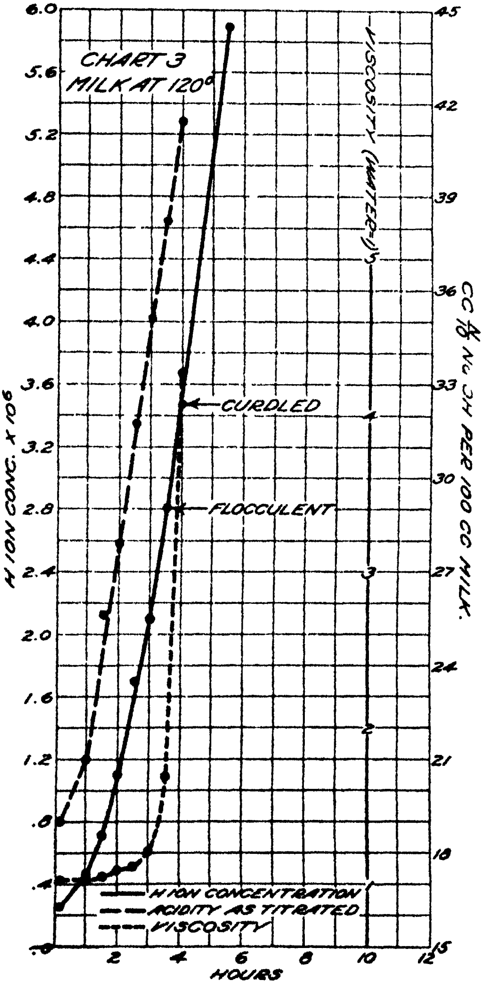

FIG. 3. EFFECT OF HEATING AT 120° ON TITRATABLE ACIDITY, HYDROGEN ION CONCENTRATION AND VISCOSITY OF MILK HELD IN SEALED CANS

are different, as might be expected from the fact that after the first hour carbonic acid was still included in the titration in one set of samples but not in the other. Evidently the chemical changes involving acid formation are practically identical in character in the sealed samples and in those in open containers under reflux.

It should be noted that in this experiment and in the ones following in which the heating was continued after coagulation had taken place, except for a slight disturbance at the incidence of the coagulation, the hydrogen ion concentration continues to follow the same curve after the precipitation of the casein. This appears to us to indicate that the casein is not appreciably involved in the acid formation but that the source of the acid is some constituent of the whey.

Two runs were made at 110° and two at 120°. The runs at any one temperature gave data in close agreement. Figures 2 and 3 show curves for one run at each temperature. The results of viscosity determinations are included on the plots to indicate visually the time and approximate rate of coagulation. Viscosity is plotted as the time required for a sample to pass through a capillary opening divided by the time required for the same volume of water to pass through the same opening.

Values obtained by calculation of hydrogen ion concentrations from measurements of potential differences at elevated temperatures are without a sound theoretical basis under present methods of standardization. Consequently, it is not possible to determine the hydrogen ion concentration at which milk curdles during sterilization. However, a hint as to the effect of raising the temperature of milk on its hydrogen ion concentration may be received from the fact that in both 110° and 120° experiments coagulation began at a time when the hydrogen ion concentration, as measured after cooling, was approximately 2.5×10^{-6}, or pH $= 5.60$. Cosmovici (2) has shown that at ordinary temperatures coagulation induced by acid begins at pH 5.35.

No attempt is made to give a detailed explanation of the sudden temporary arrest of hydrogen ion concentration change during the early stages of coagulation. However, it indicates a powerful buffer action that is manifested only for a brief time. In fact, determinations on the 120° run were not frequent enough to obtain even a suggestion of this feature of the curve. The continued smoothness of the curve of hydrogen ion concentration changes at 120° indicates again that the acid is produced mainly by a constituent of the whey.

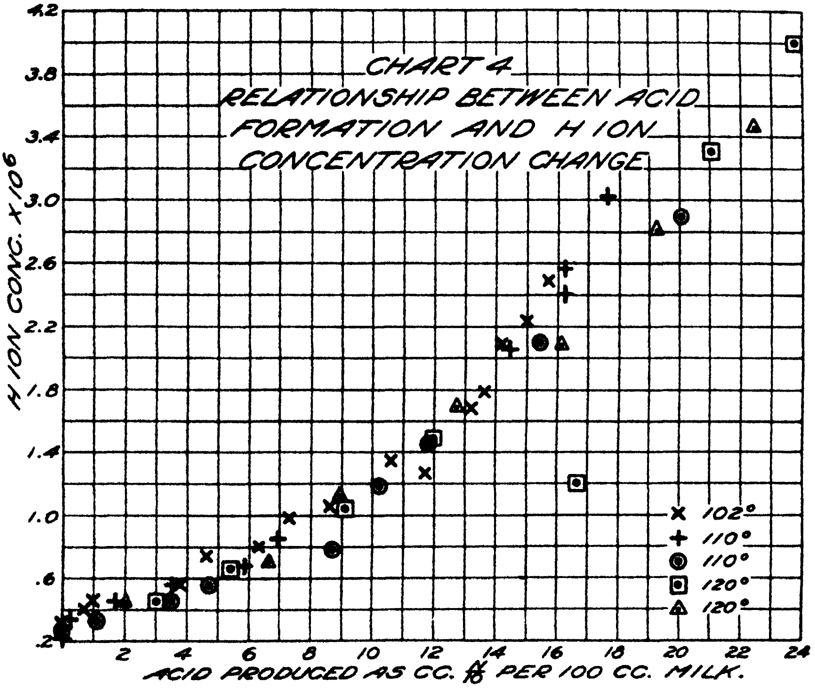

FIG. 4. RELATIONSHIP BETWEEN ACID FORMATION AND HYDROGEN ION CONCENTRATION CHANGE IN MILKS HEATED AT VARIOUS TEMPERATURES

Runs made in open flasks at 95° and 90° show nothing of interest beyond a slower rate of acid formation, and consequently the corresponding curves are not published.

The next point to be considered is the relationship existing between the quantity of acid formed by heating skimmilk and the corresponding changes produced in hydrogen ion concentration. In figure 4 is plotted this relationship for each of the five runs carried out in sealed cans. The grouping of the various points about a common curve indicates that the buffer action exerted by the milk samples was practically the same at all temperatures employed and that undoubtedly the same acids in very nearly the same proportions were formed in each case. It should be noted that of the two sets of points which stray upwards in the coagulation range, one is plotted from a run at the same temperature as another which clings closely to the flatter slope in the same range. These slight discrepancies are undoubtedly due to differences in the buffer contents of the original milks. Many workers have noted that it is unusual to find two samples of milk identical in buffer content.

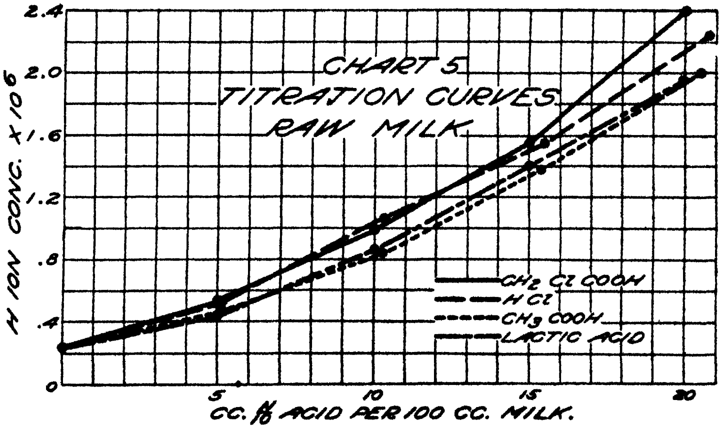

FIG. 5. TITRATION CURVES OF RAW MILK WITH VARIOUS ACIDS

The curve in figure 4 is really the titration curve of skimmilk the titrating acids being those formed during heating. If milk were titrated with an acid having a dissociation constant equal to the mean dissociation constant of the acids formed in the heated milk, a very similar curve should be obtained. It should be identical only in case a single acid is formed and the buffer action of milk is not changed by heating.

In figure 5 are plotted titration curves of raw skimmilk, acids of several different degrees of dissociation being used. Hydrochloric acid was chosen as a strong acid, monochloracetic (pK = 2.81, pK being the logarithm of the reciprocal of the dissociation constant) as an acid of medium strength, and lactic (pK = 3.82) and acetic (pK = 4.73) as weak acids. It was desired to use acids foreign to milk and of pK values in the region between 5 and 7. However, it was both disappointing and interesting to find that the only common acid radicals which have such dissociation constants are those already present in the milk.

The close similarity of the curves obtained may seem surprising at first, but it must be borne in mind that the principal buffer in milk in the range of these titrations is a mixture of phosphates and that the value of pK for the second hydrogen of phosphoric acid, which is the one principally involved, is about 7.0. Consequently, the buffer action which might be exerted by any of the relatively stronger acids used is almost completely overshadowed by the buffer action of the phosphates of milk. This curve has a much flatter slope than the group of figure 4. Since it has been observed repeatedly that phosphates are precipitated by heating milk, and since it is to be expected that the buffer action is changed by heating, titration curves were carried out on skimmilk that had been autoclaved at 120° for thirty minutes. It will be seen by a comparison of figures 4 and 6 that the curves of autoclaved milk are in good agreement with those obtained from the titration of milk by the acid produced during heating. While these findings duplicate the effect produced by heating milk in so far as the relationship of hydrogen ion concentration to acids formed is concerned, they do not identify these acids. They do, however, locate their mean dissociation constant above 1×10^{-6}. In view of the loss of buffer action caused by heating milk, objection may be raised to the above method of titration on the ground that since the amount of alkali required to change the pH of raw milk from 6.6 to 8.3 is greater than the amount that would be required after heating, the difference between the initial titration on the raw milk and the subsequent titrations on the heated milk does not give the true value for the amount of acid formed. In other words, the values for acid formed are slightly too low. However, the error must be comparatively slight and in any case would not noticeably affect the form of the time curves. The slopes of the titration curves would be quite unaffected. The primary reason for the choice of end point was that the results might be compared with those of other investigators along similar lines who have used phenolphthalein as the end-point indicator. It should be pointed out that the removal of buffer by heat is not a quickly completed reaction even at 120° but that it involves a time factor.

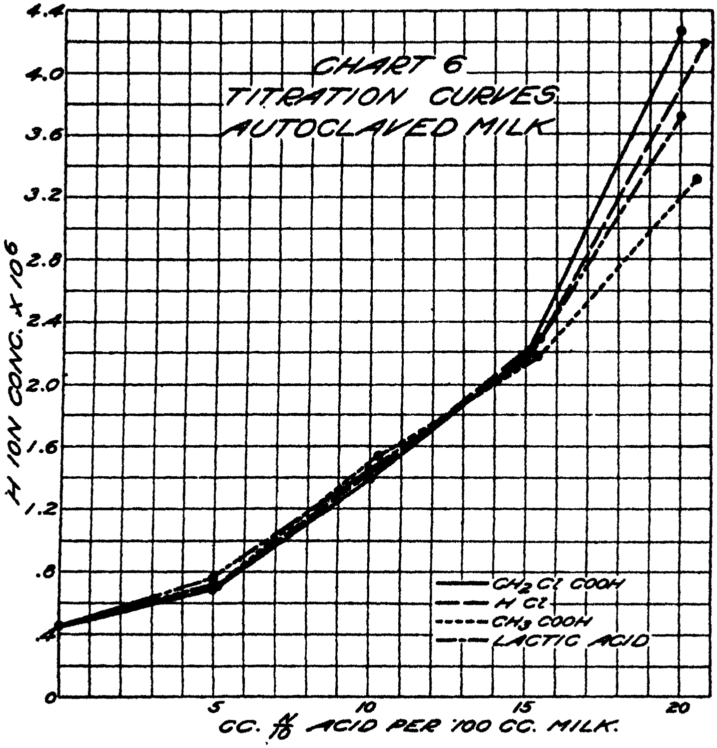

FIG. 6. TITRATION CURVES OF AUTOCLAVED MILK WITH VARIOUS ACIDS

Reasons have been stated for believing that the acid of heated milk is produced from a constituent of the whey. Investigators of the mechanism and conditions involved in sugar oxidation have found that lactose is very easily oxidized, whether in acid or alkaline solutions, to give mixtures of acids. Under weakly oxidizing influences, formic is always one of the acids produced (3). Levulinic acid is characteristic of acid oxidations of lactose;

saccharinic acids, of alkaline oxidations. In the case of heated milk, if lactose is the source of the acid formed, the rate of acid production should be a function of the lactose concentration as well as of the time and temperature of heating. To settle this point, experiments at boiling temperatures were carried out on a sample of skimmilk and a sample of the same milk in which 5 per cent lactose had been dissolved. The heating was carried out in open flasks under reflux condensers, both flasks being heated in the same bath. The rates of change of hydrogen ion concentration and acidity are shown in figure 7. Practically twice as much acid is produced in the milk containing 5 per cent added lactose,—altogether double the normal quantity,— as is produced in the normal milk. Similar experiments were also carried out on 5 per cent lactose solutions containing the same concentrations of total

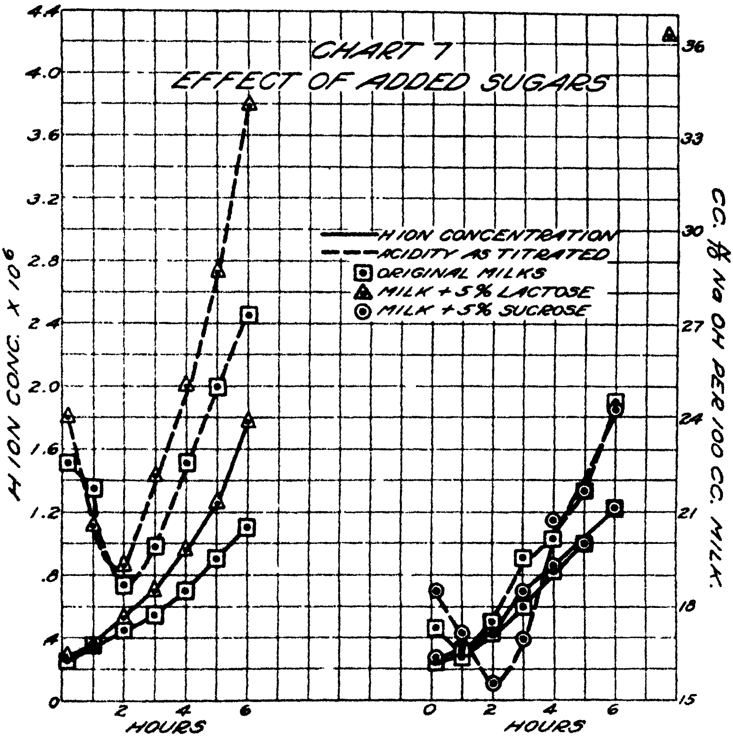

FIG. 7. EFFECT OF ADDED SUGARS ON ACID FORMATION IN HEATED MILK

phosphate, of total citrate, and of hydrogen ions as are present normally in milk, and changes in hydrogen ion concentration and acidity were obtained very similar to those in milk under the same time and temperature conditions of heating. This is considered sufficient proof that lactose is the principal source of the acid formed in heated milk.

The second set of curves in figure 7 is from an experiment analogous to the one on which the first set is based, except that a different sample of milk was taken and the sugar used was sucrose. Evidently, from the standpoint of the condensed milk manufacturer, sucrose is a much more desirable carbohydrate in milk than is lactose, since it does not produce acid during the heating of milk. The suitability of sucrose as a preservation for condensed milk is made evident by these data.

Lactose analyses were made on a sample of milk before and after heating and showed that during the production of acid equivalent to a concentration of 0.02 molal, lactose diasappers in amount equivalent to a concentration of 0.085 molal. In other words, even on a mol to mol basis, the lactose loss is sufficient to account for more than four times the amount of acid formed.

Another portion of heated milk was subjected to steam distillation. The distillate was acid and gave the characteristic tests for formic acid, which is an oxidation product characteristic of carbohydrates, but not of proteins.

Experiments on lactose solutions containing radicals similar in their buffer action to those in milk, but chemically different, would settle the question of a specific phosphate effect on reactions involving lactose. However, the only buffers that meet these requirements are certain complex organic acids, few in number and difficult to obtain. Aside from the variation in rate of color development with phosphate content, nothing in our experiments indicates any specific action of phosphate ions. However, this is as readily explained on the basis of the buffer action of the phosphate, since a continued low hydrogen ion concentration is particularly favorable to caramelization of lactose. Until it is shown that buffering acid radicals other than those present in milk, but having practically the same dissociation constants, do not exert the same physical and chemical effects on the equilibria, we feel that any claims for specificity of action of phosphates in milk are unproved.

Among the applications of the foregoing may be noted its bearing on the forewarming of milk previous to condensing. It is well known that the stability of milk during condensing and sterilizing is considerably affected by preheating. Certain times and temperatures of heating render the milk more stable; others have the opposite effect. Evidently there is a balancing of factors, opposing in effect, both sets of which are to a considerable degree functions of time and temperature of heating. The above data show that the unstabilizing influences are, at least in part, the formation of acid and the removal of buffer substances during the heating. Attainment of the most successful forewarming conditions depends upon the proper choice of time and temperature to give the most advantageous balancing of the stabilizing effect, whatever may be its character, against these deleterious influences.

Both the acid formation and the loss of buffer are factors that should be considered in modifying milk for infant feeding when previously heated milks are used. Other applications of the data here presented will occur to workers on specific problems of the dairy industry.

RECAPITULATION

Acid is formed during heating of milk at a rate which is a direct function of the time and temperature of heating and of lactose concentration.

The rate of acid formation appears to be unchanged by the coagulation of the casein.

The changes of hydrogen ion concentration and of acidity of heated milk caused by this acid production are of the same order of magnitude as those produced by heating similarly a 5 per cent lactose solution buffered with the same amount of phosphate and citrate and adjusted to the same initial hydrogen ion concentration as those existing in milk.

The relationship between the amount of acid present at any time and the hydrogen ion concentration existing at the same time in the heated milk is comparable with that established by acids with dissociation constants greater than 1×10^{-6}.

Increasing the lactose concentration of milk causes an increased amount of acid to be formed when the milk is heated. Addition of sucrose has no apparent effect on the production of acid in milk by heat.

The loss of lactose content in a heated milk is more than sufficient to account for the acid production, even on a mol to mol basis.

We conclude that lactose is the principal source of the acid produced by heating milk.

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LACTOSE SOLUBILITY AND LACTOSE CRYSTAL FORMATION

II. LACTOSE CRYSTAL FORMATION*

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It was pointed out in Part I of our investigation on "Lactose solubility and lactose crystal formation" (1) that the milk colloids had no noticeable effect on lactose solubility but that sucrose solutions decreased the solubility of lactose to some extent. This decrease was but slight in dilute solutions but became more pronounced as the sucrose concentrations increased.

These findings have a direct, practical bearing on the control of sandiness in ice cream and the control of sandiness and prevention of sediment in sweetened condensed milk. In this work it had been observed that the sugar crystals in sweetened condensed milk which, when it leaves the vacuum pan, normally represents a supersaturated lactose solution containing a sucrose-in-water concentration of about 60 to 64 per cent, were conspicuously different to the eye from the general appearance of lactose crystals as they crystallize from pure aqueous solutions. The latter are of the usual slender tomahawk shape while those found in sweetened condensed milk are distinctly pyramidal and very blunt.

These facts suggested the possibility of the formation, in solutions containing both lactose and sucrose, of mixed crystals of both sugars and of a possible relation between the effect of sucrose on lactose solubility and the form of lactose crystals. These observations, therefore, prompted a study of the crystallography of lactose crystals precipitated from diverse solutions and mixtures, such as pure lactose-in-water solutions, lactose-in-milk, lactose-in-sucrose solutions of high and low concentrations, unsweetened condensed milk and sweetened condensed milk.

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METHOD USED FOR STUDYING LACTOSE CRYSTALS

Materials used and preparation of solutions

The materials used in this investigation consisted of Mercks c.p. lactose, Difco c.p. lactose, Pfanstiehl c.p. sucrose, Domino brand sucrose, whole milk, skimmilk, distilled water, unsweetened condensed milk and sweetened condensed milk. The sucrose-in-water and sucrose-in-milk solutions were so prepared as to have present in the water part of the solution either 14 per cent or 62 per cent of sucrose prior to the addition of the lactose.

All solutions, except the condensed milks, were supersaturated with lactose by dissolving from 30 to 60 grams of lactose powder in 100 cc. of the water part of the solution at a temperature of about 150°F. in the water bath, then cooling to 65°F. and room temperature, respectively, at which temperatures the lactose was allowed to crystallize.

In a few cases crystallization was hastened by seeding the cooled solution with a definite amount of seed material (1 cc.) prepared by rapidly chilling with constant stirring a supersaturated solution of lactose in water. In this way crystallization was always assured and commenced in a much shorter time than when no seed material was added. The seed material had no effect whatever on the crystal form obtained. When using the same amount of the seed material in plain lactose-in-water solutions and lactose-in-sucrose solutions containing 14 and 62 per cent sucrose, respectively, the type of crystals distinctly corresponded with the crystal forms typical for the solution used. However, in general the use of seed material was avoided so as to preclude the possibility of influencing crystal form.

In most cases crystallization was allowed to proceed undisturbed, i.e., without agitating the solution, so as to avoid all danger of breaking or mutilating the crystals.

Sweetened and unsweetened condensed milk were used exactly as obtained from the condensery and without further modification, since these products already contained lactose in supersaturation at the temperature at which their crystal content was studied.

Preliminary crystallization studies showed that there was no advantage in using c.p. sucrose over filtered solutions of Domino brand granulated sucrose. Results obtained with Difco and Mercks lactose also were identical. For this reason the less expensive materials, i.e., Domino sucrose and Mercks lactose were used.

Examination of crystals under the microscope

A drop of the crystalline deposit was transferred to a microscope slide by means of a pipette at intervals during the development of the crystals, observing the progress of crystallization. In addition to this some of the supersaturated solutions were placed on slides and the cover glasses sealed down with vaseline to prevent evaporation. In this way the growth of individual crystals was followed.

The examinations were made under magnifications of 40×, 100×, 440× and 900×.

In studying the crystals under the oil immersion advantage was taken of the fact that by using a certain grade of immersion oil the cover glass could be lifted slightly by regulating the micrometer screw on the microscope, thus causing the crystals to move about in the solution. By careful manipulation of the micrometer screw it was possible to have the crystals turn completely over, thus permitting study from every conceivable angle. This method was of great advantage in studying crystals of as complex a form as that of lactose, since in certain positions their appearance was very misleading.

Photographing the typical crystals

In order to permanently record and illustrate the form of the crystals typical for the various lactose solutions, microphotographs were prepared.

In the case of lactose no difficulty was encountered in obtaining individual crystals. In the case of sucrose the crystals grow together rapidly, making the isolation of perfect individual crystals difficult. In order to avoid this a supersaturated solution of sucrose-in-water was prepared, and rapidly cooled in ice water with constant stirring, not permitting the crystals to unit. This method avoided the use of foreign substances to increase the viscosity for the purpose of securing individual crystals, which substances might effect a change in the crystal form.

LACTOSE CRYSTALS FROM WATER SOLUTION

Lactose crystals were studied by Schabus (2) as early as 1855 who, however, assigned them to the rhombic hemihedral system. Wulff (3) in a later study reported that they belong perhaps to the monoclinic system while Traube (4) in 1891 described them as being definitely monoclinic. This classification has been found correct by later investigators, as is also shown by Groth (5) in his extensive treatise on "Chemical crystallography" which describes lactose crystals as being monoclinic sphenoidal.

A further study of lactose crystals was made by Hashimoto (6) in 1907 and by Sato (7) in 1914. Sato worked with sweetened condensed milk and reported that the lactose crystals present did not have the same shape and appearance as those obtained when lactose crystallizes from water, milk, and milk serum solutions.

According to the newer method of crystal classification the fully developed lactose crystal belongs to class C_2 since it is enantiomorphous (not identical with its mirror image) and has one binary axis passing through base and apex. The lactose crystal has no center of symmetry and no plane of symmetry, being of a very low order of symmetry.

In their early stages of development these crystals appear as very thin triangles with an extremely small portion of their apex cut off, i.e., they appear trapezoidal. These truncated triangles grow larger in area and thicker, showing a rhomboid base parallel to a barely perceptible rhomboid surface at the apex. As the crystals grow, their base begins to bevel off giving the appearance of a tomahawk and later on the apex assumes a somewhat beveled form also. In the full stage of development the crystal has ten faces. The six sides are trapezoidal, and the base and apex rhombic. In many crystals the angle of the bevels, or M faces, is such as to seemingly eliminate the faces of the base and apex.

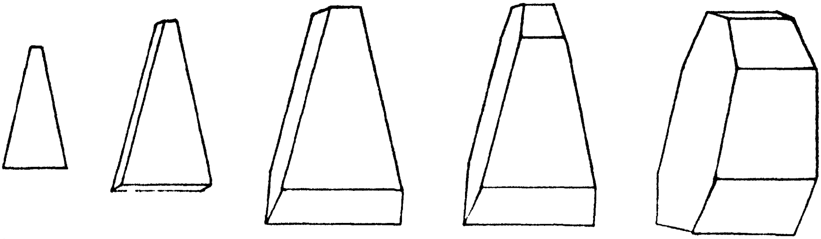

Fig. 1. Lactose Crystals from Lactose-in-Water Solutions Showing Order of Development of the Crystal as Presented from Left to Right

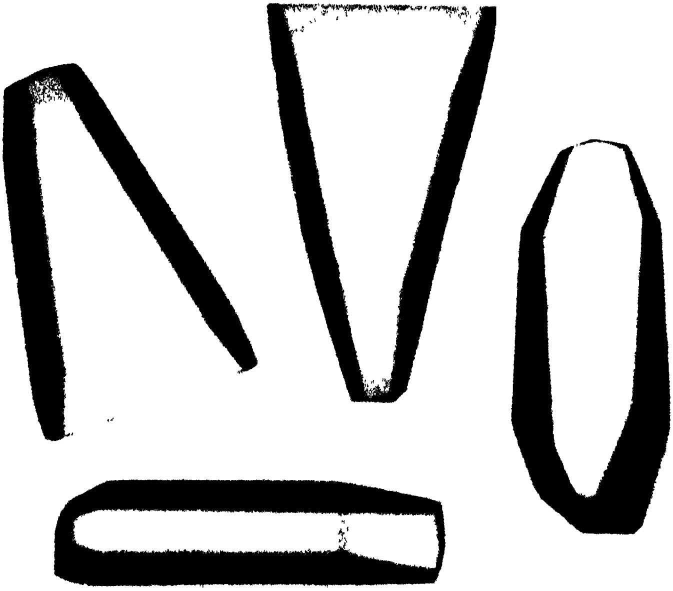

Fig. 2. Lactose Crystals from Lactose-in-Water Solution

In our study of lactose crystals from water solutions this type of crystal with the distinct tomahawk end predominated.

In the early stages of development the relation of length to width of the crystals appeared from our measurements to be 1.64

to 1, and their thickness about one-fifth of their length. In the fully developed crystal this ratio was 1.8 to 1 and their thickness about one-third of their length. However, the habit of the crystals obviously may alter these ratios slightly. These measurements are recorded here for convenience of later comparison with lactose crystals from sucrose solutions.

A sketch showing the successive stages in the development of lactose crystals from lactose-in-water solutions is illustrated in figure 1. Microphotographs of fully developed lactose crystals from the same solutions are shown in figures 2 and 3.

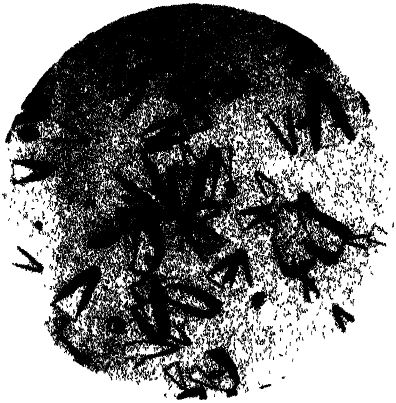

Fig. 3. Lactose Crystals from Lactose-in-Water Solution

LACTOSE CRYSTALS FROM MILK

By supersaturating whole milk and skimmilk, respectively, with powdered lactose and allowing crystal formation to take place at room temperature, the usual type of tomahawk lactose crystals, characteristic of crystals from pure lactose-in-water solutions were obtained. The same was true of plain condensed milk (unsweetened) containing from 34 to 36 per cent total milk solids and that had been held in the cooler at about 35°F. for a week or longer after being drawn from the vacuum pan. In such condensed milk the lactose is present in a state of supersaturation at the above temperature.

The crystals in both, the fluid milk and the condensed milk, were fully developed at both ends and identical in every respect to those separated from pure lactose-in-water solutions, as illustrated in figures 2 and 3. Their length also averaged about 1.8 times their width at their widest portion, as is characteristic of typical lactose crystals from pure water solutions.

It is interesting to note also that the lactose crystals from condensed whey shown by Zoller and Williams (8) are identical to our lactose crystals from pure water solutions.

These observations clearly show that solutions containing milk solids yield typical lactose crystals, and that the crystal form is in no way modified or altered by the presence in the lactose solution of milk colloids and milk fat.

LACTOSE CRYSTALS FROM SUCROSE SOLUTIONS

In our study of lactose crystals from solutions containing sucrose, we persistently obtained crystals of a form apparently quite different from the typical lactose crystals derived from lactose-in-water solutions. This study embraced sucrose solutions containing 14 per cent sucrose, 62 per cent sucrose and supersaturated sucrose solutions. The modification of the crystal form became more pronounced as the sucrose concentration increased.

In 14 per cent sucrose solutions the lactose crystals were shorter, decidedly thicker and many of the crystals lacked the beveled faces of fully developed typical lactose crystals from pure water solutions. Figure 5 illustrates the predominating crystal forms usually found to precipitate from 14 per cent sucrose solutions.

Zoller and Williams (8) studied lactose crystals from sandy ice cream containing 14 per cent sucrose and report "the sandy crystals to be identical in form to those of pure lactose." A close study of the crystals illustrated by these investigators, however, reveals unmistakably that the sucrose did have a modifying effect on the form of crystals obtained analogous to our findings with lactose crystals from 14 per cent sucrose solutions.

In the case of 50 per cent, 62 per cent and saturated sucrose solutions, the lactose crystallized out in the form of rhomboidal, pyramidal crystals. As the sucrose concentration increased the lactose crystals appeared to become shorter and broader. The crystals started as rhomboids and built up from the rhomboid form into truncated pyramids rather than from the triangular form as was observed with crystals from lactose-in-water solutions. Figure 4 is a sketch of the successive stages in the development of lactose crystals from concentrated sucrose solutions. Figures 6, 7, and 8 are microphotographs of the lactose crystals as found in these solutions.

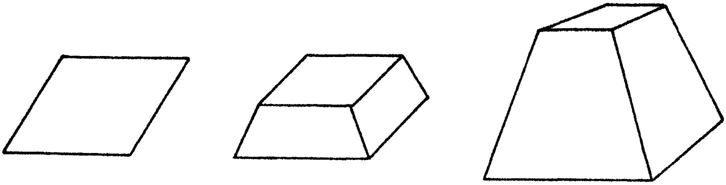

FIG 4 LACTOSE CRYSTALS FROM SOLUTIONS CONTAINING HIGH CONCENTRATION OF SUCROSE SHOWING ORDER OF DEVELOPMENT OF THE CRYSTAL AS PRESENTED FROM LEFT TO RIGHT

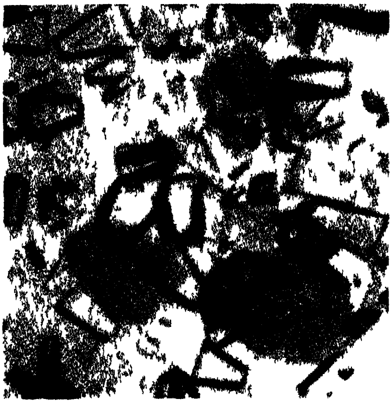

FIG 5

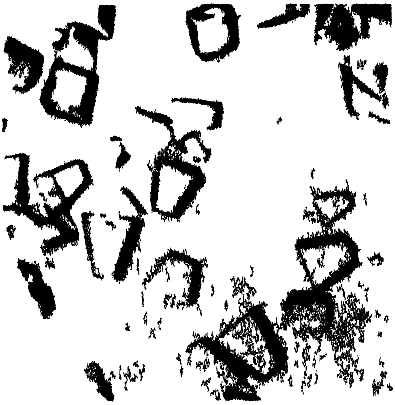

FIG 6

FIG 5 LACTOSE CRYSTALS FROM 14 PER CENT SUCROSE SOLUTION
FIG 6 LACTOSE CRYSTALS FROM 62 PER CENT SUCROSE SOLUTION

Thus it was repeatedly observed that where identical concentrations of lactose were present in different bottles containing water and 14 and 62 per cent sucrose solutions, respectively, typical lactose crystals formed in the pure water solutions and the pyramidal forms developed in the 62 per cent sucrose solutions. In the latter case the length, width and height of the crystal were very nearly equal. In the 14 per cent sucrose solution the crystal showed the typical lactose form but it was generally quite modified in the direction of a blunt type.

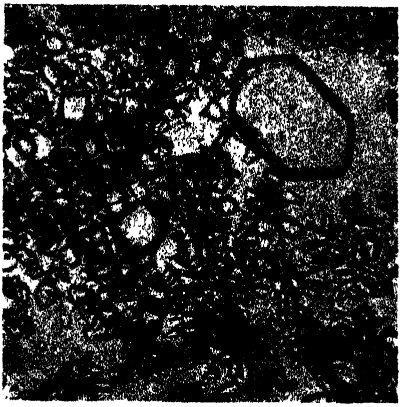

FIG. 7

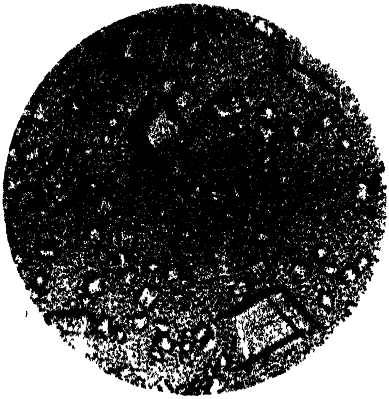

FIG. 8

FIG. 7. LACTOSE AND SUCROSE CRYSTALS FROM SUPERSATURATED SOLUTION OF BOTH SUGARS
FIG. 8. LACTOSE CRYSTALS IN SANDY SWEETENED CONDENSED MILK

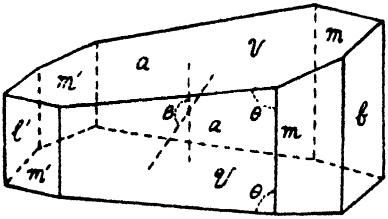

FIG. 9

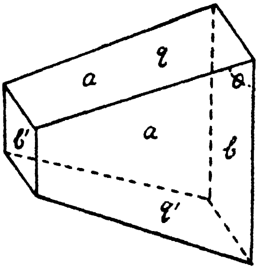

FIG. 10

FIG. 9. LACTOSE CRYSTAL FROM LACTOSE-IN-WATER SOLUTION (BY SATO)
FIG. 10. LACTOSE CRYSTAL FROM LACTOSE-IN-SUCROSE SOLUTION (BY SATO)

It was also noted in one such experiment that during a like period of time the crystals from plain water were very large, some having attained 3 mm. in length, averaging 1.5 mm. From the 14 per cent sucrose solution the average length was 0.5 mm.

The 62 per cent sucrose water solution contained a mass of very small lactose crystals averaging about 0.05 mm.

These observations indicate clearly that the presence of sucrose in supersaturated lactose solutions has a distinct modifying effect on the resulting lactose crystals and that the effect becomes more pronounced as the sucrose concentration increases, causing the triangular form to yield to the rhomboid form and the ratio of length to width to height to change to nearly 1.

Although these crystals appear to differ very materially from those derived from lactose-in-water solutions, crystallographically they belong to the same system, since they too are monoclinic sphenoidal or wedge-shape, and likewise have only one binary axis perpendicular to the rhomboid base and rhomboid top. This pyramidal form of the lactose crystals from sucrose solutions may be considered equivalent to the fully developed crystals, but lacking the beveled faces, or M faces, which are characteristic of lactose crystals from water solutions.

It appears, therefore, that the presence of sucrose affects the habit of the lactose crystal and does not permit it to assume its fully developed form. Sato (7) likewise found that the crystals resulting from crystallizing milk sugar from sucrose solutions were predominatingly of the rhomboid, pyramidal type. Drawings of lactose crystals obtained from aqueous solutions without and with sucrose are shown in figures 9 and 10, respectively.

SUCROSE CRYSTALS FROM WATER- AND LACTOSE-SUCROSE SOLUTIONS

The predominating sucrose crystal has eight faces, with all opposite faces parallel to each other. Base and top are six-sided. All sides of the crystal are rhomboid and its edges and points are often beveled off as illustrated by microphotographs in figures 11 and 12. In contrast to this the typical lactose crystal from highly concentrated or supersaturated sucrose solutions is a truncated pyramid with rhomboid base and apex.

Browne (9) points out that sucrose crystals are capable of assuming a variety of forms. The general description above given refers to our observations in the study of sucrose crystals from water solutions.

Sucrose crystals grow to relatively very large size while lactose crystals were usually only from one-tenth to one-hundredth as long as sucrose crystals. There is also a persistent tendency of the sucrose crystals to grow together forming large masses of intergrown crystal aggregates, while lactose crystals show no such tendency. They rarely grow together. Where sucrose and lactose crystallize out from the same solution, such relation of size becomes very evident as illustrated in figure 7.

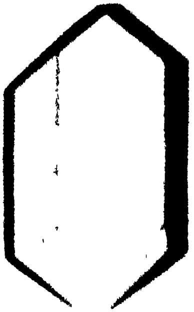

FIG 11

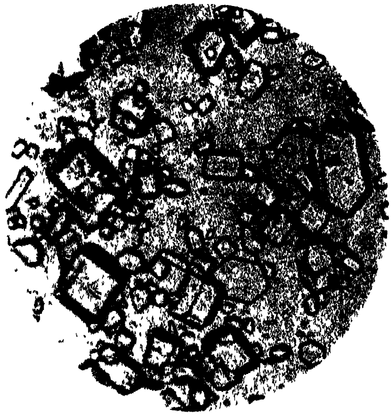

FIG 12

FIG. 11. SUCROSE CRYSTAL

FIG. 12. SUCROSE CRYSTALS FROM SUCROSE-IN-WATER SOLUTION

Our observations indicate that the sucrose crystals forming in the presence of lactose are typical of the crystals from pure sucrose-in-water solutions. It may be of interest to note here that when sucrose crystallizes in the presence of raffinose, the sucrose crystals become somewhat elongated and pointed, as shown by Browne (9). This may be considered analogous to the modifying effect which sucrose in lactose solutions has on the form of the lactose crystals as previously described under "Lactose crystals from sucrose solutions."

CRYSTALS IN SUPERSATURATED SOLUTIONS OF BOTH SUGARS

Solutions in which both sugars, the lactose and the sucrose, were present in supersaturation, were allowed to crystallize. The crystalline sediment revealed the presence of lactose crystals typical of those found in undersaturated sucrose solutions and of sucrose crystals typical of sucrose crystals from water solutions. Under the microscope these crystals appeared to be entirely independent of each other, as illustrated in figure 7 and there seemed to be a complete absence of modifications suggestive of the presence of mixed crystals of the two sugars. This is in harmony with the general observation that organic substances generally do not form mixed crystals, but crystallize out separately, each yielding crystals characteristic of that substance.

COMPOSITION OF CRYSTALS FROM LACTOSE-SUCROSE SOLUTIONS

Solutions were prepared both in milk and in water containing lactose in excess and with sucrose present to the extent of 62 per cent of the water part of the solution. The sediment of crystals forming here after a crystallization period of five to six weeks was removed by carefully draining off as much of the supernatant liquid as possible and then washing the crystals with ice cold water on the Büchner funnel several times. The crystals were then collected, dissolved in a small amount of water to complete solution and dried at 100°C. in the vacuum oven. This produced the anhydride form of crystals which were weighed as such. These crystals were analyzed for lactose by the use of the gravimetric Fehling's solution method (Association of Official Agricultural Chemists (10)) and showed to contain from 99.2 to 100 per cent pure lactose. These results represent three such determinations. These findings are also supported by the work of Zoller and Williams (8) who found that crystals isolated from ice cream averaged 99.86 per cent lactose.

Since the crystals separating from these concentrated sucrose solutions appeared to be all of the same pyramidal shape and still retained this shape after washing although much of the sharp detail was removed by the wash water, it appears safe to assume that all the adhering sucrose had been washed away and that the resulting washed crystal sediment was representative of the crystal interior only and not of any of the adhering liquid.

Furthermore, analysis of the supernatant liquid showed that exactly as much sucrose was present in the water part of the solution as was added originally. This is additional evidence that the crystals formed in the concentrated sucrose solution could not have contained any sucrose and that they were not mixed lactose-sucrose crystals but were pure lactose crystals.

SUGAR CRYSTALS IN SWEETENED CONDENSED MILK

Perhaps the most comprehensive study of sugar crystals in sweetened condensed milk was that made by Sato (7) who reports that the sugar crystals in sweetened condensed milk are milk sugar. This he proves by forming the osazone of lactose with a melting point of 200°C. He further states that exactly the same type of crystals were obtained in sweetened condensed milk as the lactose crystals crystallizing from sucrose solutions.

Our observations fully agree with Sato's work. As illustrated in figures 6 and 8 the crystals of lactose present in 62 per cent sucrose solutions are identical in form to the crystals we observed in sweetened condensed milk. We further noted that the height of the pyramidal crystals depended to some extent on the sucrose content of the sweetened condensed milk; the greater the sucrose concentration, the "squattier" the crystals. No fully developed tomahawk-shape crystals were observed in sweetened condensed milk.

This agreement of form between lactose crystals in sucrose solutions and in sweetened condensed milk is in full accord with our observations of the factors affecting lactose solubility where we showed that milk colloids have no effect on the solubility of lactose and that the sucrose alone has a solubility-diminishing effect. Likewise, as shown previously in this paper, the colloids do not have any modifying effect on the type of lactose crystals obtained, but the sucrose does prevent their full development. Hence, it is the sucrose alone that causes the modified form of lactose crystal in sweetened condensed milk.

This is quite in accord with the difficulties observed in ordinary crystallizations, so often encountered in efforts to secure typical crystals, even where only traces of impurities are present. In addition the viscosity of the solution may also be a factor in modifying crystal form.

SUMMARY

1. The crystalline form of lactose was studied. The crystals were precipitated from diverse solutions and mixtures, such as lactose-in-water, lactose-in-sucrose solutions of high and low concentrations, lactose-in-milk, plain condensed milk and sweetened condensed milk.

2. Lactose crystals belong to Class C_2. They are monoclinic sphenoidal and have only one axis of symmetry. They have trapezoidal side faces and rhombic tops and bottoms. The fully developed lactose crystal has in addition beveled faces at the base and apex which may terminate in a sharp edge giving the crystal a distinct tomahawk appearance. It may have ten faces and its length is approximately 1.8 times its width at its widest portion.

3. Fully developed, typical lactose crystals, as described above, are characteristic of the crystals obtained from supersaturated lactose solutions in water, in skimmilk, in whole milk, in milk serum, and in plain condensed milk (unsweetened). It is plainly evident, therefore, that the presence in the milk solution of the milk colloids and milk fat does in no way modify the appearance of the lactose crystal obtained.

4. The presence of sucrose in the supersaturated lactose solution causes an apparent modification in the appearance of the lactose crystals. The crystals so produced are short and stubby and lack the full development at base and apex. In the case of dilute sucrose solutions, such as solutions containing 14 per cent sucrose, this change is only slight, the lactose crystals possessing the full development, but they are shorter and thicker than the crystals from solutions containing no sucrose. This is the type of crystal that occurs in sandy ice cream. In the case of concentrated sucrose solutions, such as solutions containing 62

per cent sucrose, the change in the appearance of the lactose crystal is very marked. The crystals resemble short, truncated pyramids with flat rhomboid base and apex, the beveled faces at base and apex being entirely absent. While crystallographically these crystals are the same, they are apparently not fully developed and give a different appearance, the habit of the crystals being probably the only element affected. This is the type of sugar crystal found in sweetened condensed milk.

5. In contradistinction to the lactose crystals, the sucrose crystals, while also monoclinic sphenoidal, present a vastly different appearance. They do not have pyramidal form, all their faces being rhomboid and all opposite sides generally being parallel. Furthermore, the sucrose crystals grow to relatively very large size and have a great tendency to twine together, which appears not to be the case with lactose crystals. These differences in appearance are very noticeable where lactose and sucrose are allowed to crystallize out from solutions supersaturated with both sugars. Such solutions yield typical crystals of each sugar independent of each other.

CONCLUSIONS

The results of the investigation discussed herein suggest the following conclusions:

1. The presence of milk colloids in saturated lactose solutions does in no way interfere with the full development of the lactose crystals and such solutions yield crystals identical to those obtained from lactose in pure aqueous solutions.

2. The presence of sucrose in saturated lactose solutions has a modifying effect on the appearance of the lactose crystal.

3. For above reasons the modified appearance of the sugar crystals found in sweetened condensed milk is due to the presence of sucrose and not to the milk colloids contained in this product, the sucrose apparently preventing full lactose crystal development.

4. The crystals found in sweetened condensed milk of usual sucrose content (sucrose-water ratio below saturation) are pure

lactose crystals. They are not sucrose crystals, nor mixed crystals of both sugars.

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CHEDDAR CHEESE FROM PASTEURIZED MILK[*]

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Milk of undesirable quality is frequently delivered to factories engaged in the manufacture of cheddar cheese. This milk commonly contains an overdevelopment of lactic acid producing bacteria or other types of microörganisms which cause objectionable flavors and textures in the cheese. Such milk is often responsible for losses in the manufacturing and the curing of the cheese which would seldom occur if the milk had been of better quality.

The improvement of the quality of a milk supply under some conditions is a matter of great difficulty so that the manufacture of inferior milk into cheese is a problem often encountered. Since the common defects of cheese made from such milk are caused largely by microörganisms, it seems possible to attack the problem by pasteurizing the milk.

This application of the process of pasteurization is not new. Von Freudenreich in 1893 (8) and Fascetti in 1903 (7) were among the first to see the use of the process. In 1907, Dean (5) and Lunde and Holm (14) reported that pasteurization was not effective in improving the quality of the cheese. Liska (12) in 1912 found some improvement in the cheese made from heated milk and Dean in 1912 (6) working with camembert cheese reported that heating the milk to high temperatures improved the quality of the cheese. Other observers (2, 3, 4, 10, 20) have reported varying degrees of improvement in the cheese made from heated milk.

The outstanding work on the pasteurization of milk for cheese making in this country is by Sammis and Bruhn (17). They heated milk to 160° to 165°F. for an instant and after cooling added hydrochloric acid to the milk to stimulate the rennet

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action. Sammis (18) reported later that the process was successful in commercial practice.

In 1923, Stevenson (21) stated that the manufacture of cheese from milk heated to a temperature of 160° to 165°F. for an instant, cooled, and set with rennet, without the addition of acids or salts, was common in New Zealand. He anticipated that within a short time every factory in that country would be equipped to follow the practice.

Some observers have questioned the ability of heated milk to coagulate, with rennet extract, to form a curd suitable for the manufacture of cheddar cheese (2, 3, 9, 11, 14, 17, 19). Others have claimed that the coagulability of the milk is not injured by heating to moderately high temperatures (8, 12, 20). Rupp (16) reported that milk heated to temperatures up to 149°F. coagulated more rapidly than before the heat treatment.

PRELIMINARY EXPERIMENT

Observations were made in this study to determine the rate of coagulation of heated milk with rennet extract. Typical results of these experiments are presented in table 1. The data obtained from two samples of milk are shown. One sample was pasteurized by the flash method and the other by the holder method at the several temperatures and time intervals indicated.

The flash method of pasteurizing has a tendency to delay the coagulation of the milk while the holder method of pasteurizing appears to accelerate very slightly the rate of rennet action when the temperatures of pasteurization approximate 60°C. Since the curd produced from the milk pasteurized by the holder method resembles raw milk curd more closely this method of pasteurizing was used in this study.

EXPERIMENTAL

Method of procedure

In the manufacture of the cheese the usual precautions were taken to have identical milk in each vat. One vat of cheese in each experiment was made from raw milk and every vat of milk was made into the best cheese possible. This fact is of great importance due to the wide variations in the behavior of raw and pasteurized milk during the cheese making process.

Student's method (22) of interpreting experimental data is used extensively in the discussion which follows. By this method the mean of the differences between paired experiments, in this case the cheese made from the vat of raw milk and the cheese from a vat of milk accorded some other treatment, is calculated. The odds against any such difference occurring due to chance alone are determined from tables published by Student (23) and later extended by Love (13). These odds are stated numerically as 99 to 1 or 500 to 1, etc.

TABLE 1

The rate of coagulation of heated milk with rennet at 30°C.

TEMPERATURE OF HEATING	DURATION OF EXPOSURE	ACIDITY OF MILK	TIME OF COAGULATION	
°C	*minutes*	*per cent*	*minutes*	*seconds*
Raw	None	0 15	3	50
50	1	0.15	4	20
66	1	0.15	4	40
77	1	0.15	5	30
Raw	None	0.175	4	5
55	30	0.175	3	55
60	30	0.175	3	30
65	30	0.175	3	40

The cheese were scored at intervals during the ripening process by Mr. Horace A. Rees,[1] Professor Walter W. Fisk,[2] Mr. Robert Kirkland,[1] Mr. A. Hargrave and the author.[2] The scores of the judges are interpreted by the ratio method (15). By this method the lowest scoring cheese in a series examined by any judge is assigned a value of 0 and the highest scoring cheese in the same series is assigned a value of 100. The ratio score of a cheese examined by a judge is a numerical value which may be calculated from the formula $\frac{100\,(s-m)}{r}$; when s is the actual score placed upon the cheese by the judge, m is the score of the poorest cheese of the lot examined by the judge, and r is the difference in points of actual score between the best and the poorest cheese which the judge examined.

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[1] New York State Department of Farms and Markets.

[2] New York State College of Agriculture, Cornell University.

The effect of the heat treatment of the milk on the quality of the cheese.

It was assumed that variations in the treatment of the milk with heat affect the quality of the cheese made from it. This hypothesis was tested by dividing the milk into three identical portions. One portion was not heated; the second was heated to 145°F. for thirty minutes; and the third portion was subjected to a heat exposure whose influence on the quality of the cheese was to be compared with the treatments given the first and second portions of the milk. The results of these trials are presented in table 2.

TABLE 2

The average differences between the ratio scores of cheese made from raw, pasteurized and heated milk

HEAT TREATMENT		HEATED MILK CHEESE SCORE MINUS PASTEURIZED MILK CHEESE SCORE	HEATED MILK CHEESE SCORE MINUS RAW MILK CHEESE SCORE
Temperature	Held		
°F	*minutes*		
130	30	−9.4	+0.2
135	15	−15.4	+0.5
135	30	−6.9	+8.9
140	15	−5.1	+5.2
140	30	−0.8	+10.9
145	15	−4.8	+12.4
150	15	−2.2	+15.8
150	30	−6.4	+21.6
155	15	−18.7	−3.5
155	30	−15.2	+4.3

The cheese made from milk subjected to every variation of of heat exposure, show a tendency to be better than the raw milk cheese, and the greatest improvement seems to be in those cheese made from the milk whose heat exposure before setting most closely resembled 145°F. for thirty minutes. When these same lots of heated milk cheese are compared with those cheese made from milk pasteurized at 145°F. for thirty minutes it is apparent that their quality is not as good except when the heat treatment approximates 145°F. for thirty minutes. These data would seem to indicate that heating the milk to 145°F. for thirty minutes produces better cheddar cheese than any other holder method of pasteurization.

The term "pasteurization" will be used in the remainder of this discussion to indicate the process of heating milk rapidly to a temperature of 145°F.; holding at that temperature for thirty minutes with sufficient agitation to prevent the formation of any scum or pellicle on the surface of the milk; and then cooling as rapidly as possible to 88°F. or below.

TABLE 3

The distribution of the ratio scores of the cheese made from raw and pasteurized milk

SCORES (CLASS)	PER CENT OF CHEESE IN THE CLASS	
	Raw	Pasteurized
20 to 30	3	0
30 to 40	3	0
40 to 50	16	0
50 to 60	28	15
60 to 70	31	20
70 to 80	19	50
80 to 90	0	15

The effect of pasteurizing milk on the score of the cheese

There were 46 pairs of cheese among those made in these experiments in which one cheese of each pair was made from raw milk and the other from identical milk which had been pasteurized. The difference between the paired ratio scores of these two types of cheese shows that the pasteurized milk cheese is 14.64 points better than the raw milk cheese, and

Student's method of interpreting the data indicates that the odds against any difference as great as this occurring due to chance alone are more than 10,000 to 1.

The scores of these cheese are arranged in table 3 to illustrate the variations in quality found in each type of cheese.

It is apparent from this table that there is a tendency for the pasteurizing process to produce cheese which is not only better but also more uniform in quality than the raw milk cheese.

The improvement in the quality of the cheese due to the pasteurizing process is also shown by the mean scores of the two types of cheese. The raw milk cheese average ratio score is 58.46 ± 1.51, while the pasteurized milk cheese has a mean ratio score of 71.72 ± 0.95. The fact that the difference between the mean ratio scores does not equal the mean of the differences in score of the paired observations is due to the fact that one lot of raw milk cheese sometimes served as check for more than one lot of pasteurized milk cheese in the course of the experiments.

Variation in cheese quality during the ripening period

The cheese were arranged in groups whose ages were approximately the same, and were scored when two, six and twelve months of age. The mean scores of both types of cheese at these ages are presented in table 4. This table also shows the coefficients of variation of these scores.

The pasteurized milk cheese scores in table 4 show a variation of 5.8 points during the ripening period. Over the same length of time the raw milk cheese scores show a decline in the quality of the cheese of 13.2 points of the ratio score. The coefficients of variation in this same table show that there is a tendency for the quality of both types of cheese to become more variable as the ripening period is extended. The pasteurizing process seems to improve the keeping quality of the cheese.

A more significant comparison of the relative keeping quality of the cheese can be made by determining the mean of the differences in score between the pairs of raw and pasteurized milk cheese after intervals of two, six and twelve months in the curing rooms. At two months of age the mean difference in the ratio scores between the samples made from identical milk is 11.01 points; at six months the difference is 15.26 points, and at twelve months 18.29 points. At each interval the pasteurized milk cheese is the higher scoring of the pairs. The odds against any differences as great as these occurring due to chance alone are more than 9999 to 1. These comparisons appear to emphasize the tendency indicated in table 4 for the pasteurizing process to improve the keeping quality of the cheese.

TABLE 4

Average ratio scores of the cheese during ripening

AGE	TREATMENT	MEAN SCORE	COEFFICIENT OF VARIATION
months			
2	Raw	66 49 ± 1 69	18 5
	Pasteurized	75 71 ± 0 97	11 2
6	Raw	57 28 ± 2 15	30 5
	Pasteurized	69 89 ± 1.31	18 6
12	Raw	53.30 ± 2 66	29 6
	Pasteurized	71 46 ± 2 48	24 6

The influence of the quality of the milk on the score of the pasteurized milk cheese

When the ratio scores of the paired lots of cheese were studied it was noticed that the difference between the raw and pasteurized milk cheese seemed greatest when the raw milk cheese was of inferior quality. To verify this observation the coefficient of correlation (1) was determined between the raw milk cheese scores and the gain in score of the cheese made from identical milk after pasteurizing. This coefficient was found to be −0.54 ± 0.07 which indicates that as the score of the raw milk cheese decreases the difference in score between it and the pasteurized milk cheese becomes greater.

The coefficient of correlation between the scores of the raw milk and the pasteurized milk cheese was found to be + 0.63 ± 0.06, showing that as the score of the raw milk cheese decreases the score of the pasteurized milk cheese also decreases.

Clean raw milk which is free from undesirable flavors and bacteria can be made into good quality cheddar cheese. Milk of inferior quality on the other hand will usually produce an inferior grade of cheese. As a general rule, therefore, the score of a raw milk cheese is an approximate indication of the quality of the milk from which it was made. If this is true it may be inferred, from the correlation of the score of the raw milk cheese and the gain in score of the pasteurized milk cheese made from identical milk, that pasteurization of milk for cheese making is most effective when the raw milk is of inferior quality. From the correlation of the scores of the raw and pasteurized milk cheese it may be assumed that milk of inferior quality, even though it is pasteurized, has a tendency to produde an inferior grade of cheese.

The influence of starter upon the quality of cheese made from pasteurized milk

The amount of starter used in the manufacture of cheese is important because of its effect on the acid development during the curd making process. Trials were made to determine the optimum amount of starter to add to pasteurized milk at the time of setting. The starter used was coagulated and about eighteen hours old from the time of inoculation. The results of these trials are given in table 5 as the mean of the differences observed between the scores of the raw and the pasteurized milk cheese made from identical milk.

It seems apparent from the data of table 5 that three per cent of starter is the optimum amount to use at the time of setting. This can be true, however, only with milk which is similar in quality and acid development to the milk used in these experiments. This matter will be mentioned again in connection with the commercial method of manufacture.

The effect of pasteurization on the yield and shrinkage of the cheese

The yield of each lot of milk is calculated on the basis of the pounds of cheese produced by 100 pounds of milk and starter in the vat at the time of adding the rennet extract. The average of the differences in yield between the paired lots of raw and pasteurized milk cheese is 0.304 pound. The odds that this mean difference is significant are more than 9999 to 1. The data in table 6 indicate that this gain in yield is a permanent gain and is not lost in shrinkage during the curing process. There is some loss of weight in both the raw and pasteurized milk cheese but it is practically the same in both types.

TABLE 5

The influence of starter on the gain in ratio score of pasteurized milk cheese

STARTER	MEAN GAIN IN SCORE OF PASTEURIZED OVER RAW MILK CHEESE	ODDS THAT GAIN IS SIGNIFICANT
per cent		
1	14.52	587 to 1
2	15.56	9999 to 1
3	24.42	3300 to 1
4	16.45	30 to 1
5	9.03	9999 to 1

TABLE 6

The yield and shrinkage of raw and pasteurized milk cheese

TREATMENT	SIZE OF CHEESE	MEAN YIELD	PER CENT SHRINKAGE
	pounds	*pounds*	
Raw	10	10.17 ± 0.049	4.8 ± 0.025
Pasteurized	10	10.58 ± 0.046	4.9 ± 0.017

The gain in yield of the pasteurized milk cheese may be caused by the retention of more of the fat, solids not fat or moisture in the cheese from pasteurized than in the cheese from raw milk. Therefore the pounds of fat, solids not fat and moisture in the cheese made from 100 pounds of milk has been calculated from the percentage composition of the cheese as determined by analysis for fat and total solids. The results of these calculations are presented in table 7.

These data, which are obtained from the analysis and yields of 28 paired experiments, indicate that the pasteurized milk cheese probably owes its increased yield to the fact that it retains more of the fat, solids not fat and moisture of the original milk than the raw milk cheese. Analysis of the whey from the pasteurized milk at dipping shows that it contains 0.079 per cent of fat less than the raw milk whey. The odds against this difference being due to chance aone are 9999 to 1. This tends to verify a portion of the data of table 7. The solids not fat in the whey from both types of cheese are so variable that the mean of the differences between the pairs is 0.05 per cent with odds of only 4 to 1 that the difference is not due to chance alone. This difference may be regarded as insignificant in accounting for the increase in the amount of solids not fat in the pasteurized milk cheese over the amount in the raw milk cheese.

TABLE 7

Fat, S.N.F. and moisture in raw and pasteurized milk cheese

TREATMENT	CHEESE FROM 100 pounds of MILK AND STARTER	MEAN FAT CONTENT	MEAN MOISTURE CONTENT	MEAN S.N.F. CONTENT
	pounds	*pounds*	*pounds*	*pounds*
Raw	10 20	3 674	3.653	2 873
Pasteurized	10.618	3.897	3 745	2.976

Commercial practice

In the commercial work carried on by the Department of Dairy Industry at the New York State College of Agriculture, some milk is made into cheddar cheese. During the summer of 1924 the milk delivered to the factory was bad flavored and dirty and developed gas and acid rapidly in fermentation tests. The same defects appeared in the cheese made from this raw milk during the early part of the season. The fat loss in the whey at dipping was abnormally high, ranging from 0.4 to 0.5 per cent.

The making process consumed about ten hours from setting to pressing.

On June 24, 5000 pounds of milk were pasteurized at 145°F. for thirty minutes. The resulting cheese was clean in flavor, and smooth, close, and uniform in body and texture. The amount of pasteurized milk made into cheese during the rest of the season totaled 1,121,629 pounds. This milk contained 3.79 per cent fat and showed a yield of 10.126 pounds of cheese per hundred of milk. It is interesting to note that for the three months previous to the period of the manufacture of cheese from pasteurized milk, there were 754,750 pounds of raw milk containing 3.68 per cent fat made into cheese with an average yield of 9.724 pounds of cheese per hundred of milk.

TABLE 8

Outline of commercial method of manufacture of pasteurized milk cheese

1. Pasteurization	145°F. for 30 minutes
2. Starter	1 to 3 per cent
3. Rennet	2 to 3 ounces per 1000 pounds of milk
4. Salt	1 5 to 2 5 pounds per 1000 pounds of milk
5. Acidity of milk when received	0 18 to 0 23 per cent
Acidity of milk with starter added	0 18 to 0 235 per cent
Acidity of whey at cutting	0 125 to 0 145 per cent
Acidity of whey at dipping	0 145 to 0 170 per cent
6. Temperature when starter is added	86° to 88°F.
Temperature at renneting	88°F.
Temperature after cooking	103° to 106°F.
7. Time from setting to cutting	15 to 30 minutes
Time from cutting to stirring	2 to 5 minutes
Time from stirring to cooking	10 to 15 minutes
Duration of cooking	30 to 45 minutes
Time from cooking to dipping	40 to 60 minutes
Time from dipping to packing	10 to 15 minutes
Time from packing to milling	60 to 150 minutes
Time from milling to salting	10 to 20 minutes
Time from salting to hooping	10 to 45 minutes
Time from hooping to dressing	25 to 50 minutes
Time from setting to pressing	5 to 6 hours
Duration of pressing	14 to 16 hours

The method of manufacture developed experimentally and practiced on the large scale is outlined briefly in table 8.

Examination of the outline of the making process should not lead to the incorrect conclusion that it is possible to make the finest grade of cheese in the minimum time allotted each operation. The time element in curd making is of secondary importance because the character of the curd determines primarily the speed of the cheese making process.

Variations in the chemical and physical characteristics of the milk make necessary certain small but important adjustments in the process. Some of these variations which are indicated in the outline may well be discussed.

1. The pasteurizing temperature seems most effective when maintained at 145°F. for thirty minutes.
2. Enough starter is added to the milk before setting to produce 0.165 per cent of acid in the whey at the time of dipping.
3. Enough rennet is needed to obtain a curd firm enough to cut in from fifteen to thirty minutes from the time of adding the rennet.
4. Curd requires enough salt to suit the trade demand. Since the yield of cheese may vary from day to day, the amount of salt is also varied.
5. The ability of raw milk to develop acid rapidly is destroyed by pasteurization. Successful curd making depends on the presence or the development of sufficient acid to bring about the normal loss of moisture from the curd. It is necessary, therefore, in order to obtain the correct acid development at dipping, to adjust the amount of starter used to the initial acidity of the milk and the time the curd is expected to be in the whey.
6. The temperature of the milk when the starter is added is near the setting temperature because usually no time interval is necessary for acid development before adding the rennet.

The setting temperature which seems most advantageous is 88°F. At lower temperatures the curd has a tendency to be tender like a very sweet, raw milk curd. Higher temperatures affect unfavorably the rate of draining of the moisture from the curd.

The cooking temperature varies with the fat in the milk, the acidity developed, and the character of the curd. The cooking temperatures of 103° to 106° seem to be desirable with pasteurized milk because of its tendency to retain moisture due largely to the slow acid development in the whey.

7. The time consumed by each operation after adding rennet depends on the character of the curd and the rate of acid development in the whey. Regardless of the time element the curd at dipping must be firm or "shotty," while the whey has preferably not more than 0.17 per cent acid or when the curd strings not more than $\frac{1}{8}$ inch on the hot iron. This desired firmness and acid development is attained in two to two and one-half hours from the time of adding the rennet.

The time allowed for cheddaring depends on the desire of the maker to produce a close textured cheese. Holding for long periods gives a close testure, shorter periods result in more open textures. Proper cooling of the curd between milling and salting, thorough distribution and solution of the salt, followed by slow application of pressure in the hoop result in better cheese than those finished by a more hasty and careless process.

CONCLUSIONS

1. Pasteurization at 145°F. for thirty minutes produces better quality of cheese than does any other method of pasteurizing tried in these experiments.
2. Pasteurized milk produces more uniform cheese, of better average quality, which keeps better under storage conditions than raw milk cheese.
3. Pasteurization is most effective when the raw milk is of inferior quality but the quality of pasteurized milk cheese varies with the quality of the raw milk.
4. Pasteurization increases the yield of cheese.
5. Manufacture on a large scale demonstrates that pasteurization of milk for cheddar cheese making is practicable, economical and profitable.

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EFFECT OF MINERAL DEFICIENCY ON THE YIELD AND COMPOSITION OF COW'S MILK*

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The relation of mineral deficiencies in the ration to the yield of milk is a question of considerable economic importance. It is also important to know from the nutritional standpoint whether the mineral elements of the milk ash—especially calcium and phosphorus—are affected by an insufficient supply of these in the feed consumed by the animal.

It has long been observed in some of the extremely mineral deficient areas of Europe and Africa that the milk yields are reduced markedly when outbreaks of osteomalacia occur. Tuff (1) noted that a decrease in milk yield was a common result of shortage of lime and phosphoric acid in the roughages fed to cows in a part of Norway. If a mineral supplement such as bonemeal or herring meal was fed, the yield of milk was greater. With cows on pasture where osteomalacia did not occur, the milk yield was satisfactory even without such mineral supplements. Theiler (2) observed that ten cows receiving a ration otherwise very deficient in phosphorous produced 40 per cent more milk when the ration was supplemented with bonemeal than did another similar group of cows without bonemeal. Weiske (3) noted that cows decreased in milk flow when receiving a ration low in calcium and phosphorous and Fingerling (4) noted the same results with goats.

Hasselbalch (5) divided a large herd of Jersey cows into two groups, one group receiving a commercial mineral mixture. This

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group yielded an average of 13 kgm. per cow more "butter" in the following one hundred and eighty days than did the controls. Two other groups of cows receiving dibasic sodium phosphate during the dry period yielded 5 kgm. more butter each than in the preceding lactation. Seven first-calf heifers excelled the yield from 9 control heifers by 4.3 kgm. of butter. He concluded that under his conditions the mineral supplement resulted in an increase of 10 to 15 per cent in butter yield. There are districts in Denmark where cattle suffer from lack of minerals in the feeds grown locally, but Hasselbalch did not state whether his herd was located in such an area.

Meigs and Woodward (6) supplemented the rations of dry cows with sodium phosphate. On comparing the milk yields between the tenth and fortieth days of the subsequent lactation with those of the previous, they noted a 37.9 per cent increase in milk yield over the expected quantity. Meigs (7) found that the decline in milk production was less rapid on a high calcium ration when supplemented with calcium carbonate and most rapid on the low calcium ration with mineral supplement.

A number of investigations are also reported dealing with the relation of the mineral content of the ration and the ash of milk. With the exception of one experiment reported in 1891 the results indicate that the mineral content of milk both in amount and percentage of constituents is not varied with the mineral intake. Among those reporting these results are Golding and Paine (8), Trunz (9), Weiske (10), Orla-Jensen (11), and Jordan and associates (12).

Most of these investigations either covered short periods or were made with cows presumably in normal condition at the beginning of the feeding period. In view of the present knowledge concerning the extent to which a cow will store mineral matter in time of ample supply and draw upon it in times of deficiency, experiments made under such conditions can hardly be looked upon as conclusive. Only in the report of the experiments by Jordan is there any evidence that the cows used were suffering from mineral deficiency to the extent of showing typical symptoms.

For these reasons it appeared worth while to present data taken in connection with an investigation of a deficiency in the rations of dairy cattle which is common in parts of Minnesota (13). The symptoms are lack of thrift, low condition of flesh, undersize, abnormal decline in milk production, stiffness in joints sometimes severe, an extremely abnormal appetite evidenced by persistent chewing of bone, eating wood and dirt. The general term osteomalacia is among those used in the literature in designating this condition. The investigations reported in full by Becker show the trouble to be the result of a shortage of phosphorus in the forage grown in the region affected due to the small content of the same element in the soil. The chief forage used is prairie hay, although the trouble occurs at times when alfalfa is fed. The soil is not acid and legumes grow freely.

EXPERIMENTAL RESULTS

The data presented were taken from four cows. The animals received a basal ration of prairie hay and oats grown on farms where the trouble has commonly occurred. Three showed the symptoms of advanced osteomalacia when purchased and the same condition was produced with the fourth animal by restricting her feed to the basal ration for several months. The cattle had free access to common salt and were watered twice daily. In all favorable weather they were allowed exercise for several hours daily in an open pen in the direct sunlight. Five-day composite milk samples were collected monthly and preserved with formaldehyde for mineral analyses. After the first series of milk samples were secured, the basal ration was supplemented with an inorganic mineral supplement, and milk samples again taken at regular intervals. With two of the cows, a phosphate supplement was later substituted for calcium carbonate, and further milk samples taken. The two animals receiving calcium carbonate as a supplement to the basal ration declined in vigor and condition indicating the deficiency was not in the calcium. Marked improvement and complete recovery from any visible symptoms of active osteomalacia was effected when the basal ration was supplemented with either mono-basic sodium phosphate or tricalcium phosphate.

Twenty-four milk samples were analyzed for total solids, total ash, phosphorous and calcium, according to the standard methods by the Division of Agricultural Biochemistry. The butter-fat content of the milk was determined by the Mojonnier method. The results of the analyses are summarized in table 1.

The data given show no indications of an increase in the calcium or phosphorous content of the milk attributable to the addition of calcium carbonate, calcium phosphate, or mono-basic sodium phosphate to a ration of prairie hay and ground oats. In three out of four instances, there was an increased milk yield attributable to the addition of a phosphate supplement to a ration low in total phosphorous.

TABLE 1

Effect of mineral supplements on the yield and composition of milk

MINERAL SUPPLEMENT FED	NUMBER OF SAMPLES	DAILY MILK YIELD	TOTAL SOLIDS	FAT	ASH	P_2O_5	CaO
		pounds	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Cow E-32:							
Basal ration..........	4	16 96	13.310	3 519	0.717	0.236	0.172
$Ca_3(PO_4)_2$.............	3	20.01	12.87	4.212	0.736	0.193	0.143
Cow E-58:							
Basal ration..........	1	3 94	16.74		0.875	0.260	0.210
$CaCO_3$...............	2	4.30	15.215	4.430	0 73	0.190	0 160
NaH_2PO_4.............	4	5 13	14.52	4.158	0 716	0.223	0.163
Cow E-59:							
Basal ration..........	1	6.36	16.210		0.875	0.275	0.202
$CaCO_3$	4	7.44	13 96	5.087	0.780	0.244	0.185
NaH_2PO_4.............	1	6.98	13.995	5.046	0.780	0.246	0.182
Cow E-62:							
Basal ration..........	2	5 68	14.538	4 87	0 785	0.235	0.182
NaH_2PO_4.............	2	6.55	14.273	4 78	0.750	0.230	0.180

The small increase in milk flow on the addition of calcium carbonate to the ration of cows E-58 and E-59 may be due to the first sample having been taken but a few days after the animals had been shipped to University Farm.

With cow E-59, a milk sample was taken made up of aliquot parts of each milking for the last ten days while calcium carbonate was being fed, and in the following ten days when she received mono-basic sodium phosphate. The nation received, exclusive of the mineral supplement, was the basal ration known to be mineral deficient. The results on the yield and composition of the milk are shown in table 2.

These data fail to show any change in the mineral content of the milk as the result of changing from the calcium carbonate to the sodium phosphate supplement.

TABLE 2

Yield and composition of milk of cow E-59 when fed calcium carbonate and sodium phosphate

MINERAL SUPPLEMENT	YIELD OF MILK	FAT	TOTAL ASH CONTENT	P_2O_5	CaO
		per cent		*per cent*	*per cent*
$CaCO_3$	69.1	5.09	0.780	0.248	0.193
NaH_2PO_4	69.8	5.06	0.780	0.246	0.182

TABLE 3

Calcium and phosphorus in milk from mineral deficiency rations compared to normal

	NUMBER OF SAMPLES INCLUDED	TOTAL ASH	P_2O_5		CaO	
			Per cent	Per cent of total ash	Per cent	Per cent of total ash
		per cent				
Check sample	1	0.717	0.215	29.9	0.170	23.6
Cow E-32	4	0.717	0.235	32.6	0.172	23.9
Cow E-58	1	0.875	0.260	29.7	0.210	24.0
Cow E-59	1	0.875	0.275	30.8	0.202	23.0
Cow E-62	2	0.740	0.235	31.7	0.182	25.1

At the time the experimental samples were prepared for analysis a sample was taken from the mixed milk of the University Herd representing approximately 1000 pounds daily produced by forty cows of four breeds. This sample analyzed with the experimental samples by the same analyst serves as an excellent check with which to compare the experimental samples. In the comparison shown in table 3 is given the analysis of the check sample together with those of the experimental samples taken from the cows at a time when they were showing marked evidences of mineral deficiency.

The data in table 3 do not bring forth any evidence that the ash of the milk from the cows suffering from extreme and long continued mineral deficiency is changed to any appreciable extent from the normal.

CONCLUSION

The data given indicate that a shortage of phosphorus in the ration extending over a long period of time may become the limiting factor in milk production. Even under conditions of osteomalacia so severe as to show marked symptoms in the animal resulting from an extreme and long continued shortage of phosphorus the calcium and phosphorus content of the milk remains normal in amount and in proportion.

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PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The twenty-first annual meeting of the American Dairy Science Association was held in the Book-Cadillac Hotel, Detroit, Michigan, October 7, 8 and 9, 1926.

The first general session was devoted to business and committee reports and a splendid address on "Science All the Way Through," by Dr. K. L. Butterfield, president of the Michigan State College.

The Secretary's report showed a substantial growth in the Association over the previous year, amounting to about 30 per cent. This places it on a sounder financial basis in the performance of its functions.

President O. E. Reed appointed the following committees:

Nomination: Borland, Davis, Fitch, Rogers, C. W. Larson.
Resolution: Guthrie, C. W. Larson, Eckles.
National Research Council: Term expiring 1928, C. W. Larson; term expiring 1929, R. S. Breed and G. C. White.

The banquet, at which three humdred were present, served as the setting for the announcement of awards in the judging contests, as usual.

The second general session was devoted to reports of sections and new business as follows:

Adoption of resolutions pertaining to the death of W. A. Stocking, an expression of appreciation to the Book-Cadillac Hotel Management, and an expression of a need for the modification of the present representation plan in force by the Division of Biology of the National Research Council.

A Program Committee of three members was appointed with the following personnel: C. H. Eckles, three years; H. A. Ruehe, two years, and C. C. Hayden, one year.

The committee on relations with the National Milk Dealers' Association was continued with one change. The members are H. A. Ruehe, E. S. Guthrie, and G. D. Turnbow.

A resolution was adopted asking the executive committee to consider the advisability of holding a summer meeting of the American Dairy Science Association in order to avoid the conflicting activities in connection with the National Dairy Show.

I. PRODUCTION SECTION

Chairman C. C. Hayden gave a brief synopsis of papers by G. C. White, E. B. Meigs, and E. B. Forbes. The new officers elected are Chairman, A. C. Ragsdale, Columbia, Mo., and secretary, H. W. Cave, Manhattan, Kansas.

II. MANUFACTURING SECTION

Chairman A. C. Baer reported for this section.

The Official Score Card for milk was amended so as to read: The official score card for milk shall give a perfect score for fat and for solids-not-fat whenever such samples comply with local requirements where contest is held.

The officers elected are Chairman, E. S. Guthrie, Ithaca, N. Y., and secretary, W. H. E. Reid, Columbia, Mo.

III. EXTENSION SECTION

Chairman A. J. Cramer presented a synopsis of the following committee reports: Milk Campaigns, Feeding, Cow Testing Associations, Bull Associations, Calf Clubs, and Dairy Manufacturing.

The officers elected are Chairman, A. J. Cramer, Madison, Wis., and secretary, C. R. Gearhart, State College, Pa.

IV. ADVANCED REGISTRY SECTION

Chairman Wylie presented the report for this section.

Changes in rules adopted follow:

1. That the supervisor should refrain from talking during the milking operation.
2. That a blank test be run on the sulfuric acid.

A resolution requesting the Breed Associations to publish the revised rules of the American Dairy Science Association in their respective handbooks. Other important papers and reports were received.

The officers elected are Chairman, P. S. Williams, State College, Pa., and secretary, J. M. Fuller, Durham, N. H.

(Signed) G. C. WHITE, *Secretary.*

PRODUCTION SECTION

The Production Section of the American Dairy Science Association held its annual meeting at 2:00 p.m., Friday, October 8, 1926 at the Book-Cadillac Hotel, Detroit, Michigan.

Chairman C. C. Hayden, presided. The minutes of the previous meeting as read by Secretary O. G. Schaefer, were approved.

G. C. White of Storrs, Connecticut read a paper on Abortion Eradication in which he emphasized the following fundamental points which have been firmly established with reference to the disease:

1. New born calves are not permanently infected by their dams, and they enter a susceptible stage only after sexual maturity is reached and particularly after they have become pregnant.
2. When the disease is once established in an individual or a herd, it is apt to be permanent, and although the animal may become capable of producing normal, living calves, it continues to harbor the infection and to spread the disease to susceptible animals which enter the herd.
3. The B. abortus germ is responsible in whole or in part for at least 90 per cent of the premature calvings occurring before the 265th gestation day.
4. The blood tests are a reliable means of diagnosing the abortion infection.

E. B. Forbes presented an interesting paper on Mineral Requirements for Dairy Cattle which he summed up as follows:

> The present evidence warrants emphasis on the use of leguminous roughage; on as much exposure of the cows as is practicable to direct sunlight; on as much use as possible of green feeds; on the curing of hay with the minimum of exposure to dew and rain, and on the allowance of a dry, resting period, and of feed during this period, sufficient to permit the complete restoration of the previous mineral losses.
>
> The present evidence warrants the use of mineral feeds only on an experimental basis, and does not warrant the inclusion of mineral components in commercial feeds.

The committee on Minerals for Dairy Cows was asked to continue its work of reviewing and summarizing the work done on this subject, in order to determine if possible, in a short period of time, what are the necessary minerals for dairy cows. The members of this committee are:

E. B. Meigs, United States Department of Agriculture
C. H. Eckles, St. Paul, Minnesota
C. F. Hoffman, East Lansing, Michigan
H. B. Ellenbarger, Burlington, Vermont
C. F. Monroe, Wooster, Ohio

"Future Coöperative Work in Mineral Nutrition" was the title of a paper by E. B. Meigs in which he said,

> In the present stage of scientific progress, work on the mineral requirements of dairy cattle should be made to center about the subject of calcium and phosphorus metabolism. Other mineral elements no doubt play a very important part in the nutrition of these animals, but in no other field is there so much promise of important practical information from a moderate amount of further work as in the subject of calcium and phosphorus metabolism.
>
> The results of the work so far carried out on this subject indicate that dairy cows, and particularly high producing dairy cows are likely to suffer from a deficiency of calcium in their rations under conditions of feeding and management which are widely prevalent in many parts of this country. They indicate further that the full effects of this deficiency are likely not to appear until after the animals have been on such rations for a long time, and are of such a character that they would not be attributed to faulty feeding, unless the connection was experimentally proved. It is of the greatest importance, therefore, that our information on the questions, what constitutes a ration deficient in calcium, what are the effects of feeding such rations, and how are these effects to be avoided, should be as definite as possible.

The following officers were elected for 1927: A. C. Ragsdale, Columbia, Mo., Chairman; W. H. Cave, Manhattan, Kansas, Secretary.

Meeting adjourned.

O. G. Schaefer, *Secretary.*

MANUFACTURING SECTION

The meeting of Section II of the American Dairy Science Association was called to order by Chairman A. C. Baer at 2 P.M., October 8, 1926 at the Book-Cadillac Hotel, Detroit, Michigan.

Minutes of the twentieth meeting were read by F. J. Doan, Acting Secretary in place of C. D. Dahle, and approved.

J. H. Frandsen reported for the committee on Score Cards and Legal Standards, to the effect that the official score card for milk should be amended to agree with the new government card used by the Bureau of Dairy Industry in which a perfect score is given for the items: fat and solids-not-fat whenever samples comply with the local requirements in respect to these ingredients.

Report was adopted and sent to general session for approval.

A. C. Dahlberg, Chairman of the general committee on Chemical Methods of Testing Dairy Products reported on the organization of this committee and its relation to the sub-committees. The committee suggested the appointment of a sub-committee to study methods of testing skim milk, butter milk and whey for fat. They further suggested that attention be given in the near future to standard methods for analysis of butter for salt, water and fat; and of cheese for total solids and fat. The report was approved.

A. C. Dahlberg reported as chairman of the sub-committee on The Comparative Accuracy of the Gerber and Babcock Tests. The two tests were found to be comparable from the standpoint of accuracy but for obvious reasons it was the opinion that one good practical test for fat in milk and cream is better than two of equal merit. The report was approved.

The report of the sub-committee on Official Methods of Testing Milk and Cream for Fat was given by Chairman F. W. Bouska. The committee first recommended the publication of a pamphlet containing the official methods of the American Dairy Science Association to be sent to all college dairy departments and government laboratories and to commercial laboratories making application.

This matter was approved subject to approval by the general session.

Recommendations were also made relative to changing the official A. D. S. A. Babcock method for fat in milk and cream in certain particulars.

After some discussion the recommendation was adopted subject to approval of general session. (Note: In general session the recommendations were not approved but were referred back to committee with suggestion that A. D. S. A. methods and A. O. A. C. methods be kept identical and that opinion of the latter organization be obtained before definitely changing the methods as they stand.)

Further suggestions of this committee favored the study of the butyl alcohol test for buttermilk, modified Babcock methods for buttermilk, skim milk and whey, and modified Babcock methods for evaporated milk.

Committee report was approved.

R. C. Fisher, Chairman of the Committee on Testing Ice Cream for Fat and Solids reported results of a survey made to determine what tests were being used for fat in ice cream, and what sort of comparative results were being had. In general, survey showed that the Glacial-Acetic-Sulphuric Acid test, the Alcohol-Sulphuric Acid test and the Glacial-Acetic-Sulphuric-Nitric Acid test were the most used. The committee is to continue its study with a view of making recommendations for standard official methods at a future date. Report approved.

The committee on Methods of Determining Milk Solids not Fat made no report.

R. S. Breed, chairman of the general committee on Bacteriological Methods reported for this committee concerning the organization and work of the various sub-committees, existing and contemplated. He also reported for the sub-committee on milk and cream, stating that this committee had the opportunity of examining the manuscript of the proposed new edition of the Official Methods of the A. P. H. A. Several changes were noted from former editions but these must be approved at the meeting to be held at Buffalo, October 1926. Report approved.

The sub-committee on Ice Cream represented by Chairman A. C. Fay reported on the drawing up of bacteriological methods for ice cream but suggested that they be published in the Journal for examination by members and discussed at a future meeting. Report approved.

Report of the Committee on College Creameries was read by the secretary. The report of this committee made last year together with the resolutions was sent to the National Ice Cream Manufacturers Association, the International Association of Milk Dealers and the American Creamery Buttermakers Association asking for a similar resolution from their respective associations. The executive committee of the milk dealers association refused to recommend the report to the association for action. The other two remain to be heard from definitely. Report approved.

R. B. Stoltz presented the majority report of the committee concerning National Contests for Judging Dairy Products. The committee favored the scoring of seven samples of each product instead of ten and the subsequent reduction of the time from one hour to forty-five minutes. They also favored the idea of the coaches being allowed to score the products during the student contest. After considerable discussion action was taken giving the committee power to draw up rules and regulations for the contest next year.

R. C. Potts, Chairman of the Committee on Economic Phases of the Dairy Industry presented a very interesting report on the experiment station Purnell projects and how they were distributed among the various agricultural departments. Report approved.

Chairman Baer appointed the following Committees:

Nominating Committee: J. H. Frandsen, R. C. Potts, H. F. Judkins

Resolution Committee: F. W. Bouska, O. F. Hunziker, M. Mortensen

W. H. E. Reid of Missouri presented a paper concerning the effect of processing on the dispersion of fat in an ice cream mix. High points in the conclusion were as follows:

Processing decreased the size of the fat globules but brought about greater clumping of the globules.

Processing increased the surface tension of the mixes.

Processing lessened the stability of the ice cream but made for smoother texture and greater control of the freezing process.

A discussion topic on the relation of sugar to overrun and quality was well presented by P. L. Lucas of Michigan.

Results of experimental work showed that sugar hinders the development of overrun but betters the texture. Excessive amounts however, slow up the freezing process due to lowering of the freezing point, produce a gummy, heavy body, make the cream hard to hold in the dealer's cabinets and lessen the standing up qualities. A sugar content of 14 to 16 per cent was deemed the optimum amount.

H. H. Sommer of Wisconsin presented a very interesting discussion topic concerning the effect of milk salts on the whipping ability of ice cream mixes which was illustrated by means of graphs.

Sodium citrate and sodium phosphate accelerate the whipping while calcium salts retard it. Neutralized cream therefore has a retarding effect. It was very clearly brought out in the graphs that viscosity is a very uncertain thing by which to judge the "whippability" of a mix.

E. S. Guthrie of Cornell presented a paper entitled "Body of Butter." His data covering a rather extensive period of observation shows that while medium worked butter is slightly superior to thoroughly worked goods in flavor (0.26 pt.), body (0.01 pt.) and hardness (0.41 pt. by the Perkins test), the advantage was all in favor of the thoroughly worked product since the composition was much more uniform and the shrinkage 28 pounds less per 1000 pounds than for the medium worked lots.

The next subject, "Uniformity of Methods of Manufacture of Cottage Cheese," was ably discussed by Carl E. Lee of the Gridley Dairy Company of Milwaukee in the short time at his disposal.

The demands for cottage cheese are so different as regards texture, firmness, acidity, etc., that no definite procedure can be given to suit all purposes. In a general way the rapidity of cooking and temperature reached determines the type of curd. The Gridley Dairy Co. has been using a light cream to mix with the curd for retail cottage cheese putting the product into glass jars handled in the regular pint milk crate. For wholesale purposes to the restaurant, hotel and baker trade etc. a single service container similar to a lard can is used and the curd is in most cases packed raw.

The time being late other topics on the program were dispensed with.

Two papers covering topics on the program were received by the secretary. J. M. Sherman, Cornell, reported on the "Preservation of Cottage and Cream Cheeses in Vacuo."

Cream and cottage cheese after storage for one year at temperatures under 50°F. in vacuum sealed jars was still palatable. This method of packaging prevents mold and even at room temperatures extends the keeping period very pronouncedly. Machinery for packing in this fashion is not materially greater in cost than present machines and the cost of operation only slightly greater.

A. C. Dahlberg, Geneva, prepared a paper covering some work in progress at the Geneva Station on cream cheese. (See page 106, this issue.)

The resolution committee presented an appropriate resolution concerning the death of Professor W. A. Stocking, Jr., late of Cornell. This was ordered presented to the general session.

E. S. Guthrie, Ithaca, New York, was selected chairman and W. H. E. Reid, Columbia, Missouri was elected secretary.

Meeting adjourned at 6:00 p.m.

F. J. DOAN, *Acting Secretary.*

DAIRY EXTENSION SECTION

The Extension Section, of the American Dairy Science Association held its annual meeting at 2 p.m. Friday, October the 8th at the Book-Cadillac Hotel, Detroit. The meeting was in charge of A. J. Cramer, Chairman, Madison,. Wis., A. C. Balzer, Vice-Chairman, East Lansing, Michigan, and C. R. Gearhart, Secretary, State College, Pa. A brief summary of reports from the five various committees follows:

1. The report of the Milk Campaign Committee was presented by Professor W. P. B. Lockwood for Miss Jessie Hoover of the Bureau of Dairying, Washington, D. C.

Milk campaigns have been successfully introduced in several states of the Union. Arkansas and Montana are two of the latest states to try this. In Hot Springs, Arkansas the consumption of milk per capita was one-fourth pint of milk daily before the campaign started. The daily consumption was just doubled in nine months. The home demonstration agent discovered that an increase of from 5 to 16 per cent in the weight of children was made in a period of 7 weeks after the amount of milk in the diet was doubled. Among the other states doing county wide campaigns are Washington and Wisconsin. The results of all milk campaign work, both rural and urban, have been highly satisfactory and it is known that the increased consumption of milk has greatly improved the health of the children in addition to the weight they have gained from it.

2. The report of the Committee on Feeding was made by C. A. Hutton of Tennessee.

The Dairy Extension men find it a big problem to get dairymen to adopt better feeding practices. And the committee men all found that the most effective way to get farmers to change their methods in feeding is through a Cow Testing Association. Next in favor after this method the feeding schools and barn meetings were found most effective. Many farmers will more readily ask questions and show more interest on the feeding subject if feeding is discussed in the barn rather than at formal meetings. It is thought that the briefly outlined feeding leaflets, circular letters and news letters, and bulletins are more effective than larger bulletins.

3. The Cow Testing Association Committee report was made by Mr. A. C. Baltzer of Michigan.

Now all states are living up to the use of Standard Uniform Cow Testing Association Rules, which were presented by the committee a year ago, receiving the approval of the American Dairy Science Association. It is recommended that the established Cow Testing Association rules be continued. In many states the C. T. A. work has increased with the additional help of more dairy specialists in the field. The results of the work have shown a marked increase in profitable 300-pound butterfat herds. The committee for next year will work on the various methods employed in training of testers, getting publicity to the Cow Testing Association members and other dairymen and on the testers conferences. The committee presented a resolution for adoption by the Extension Section, to go to the Committee on Extension Organization and Policy of Land Grant Colleges to ask the U. S. D. A. to call a national meeting in Washington, D. C., of all Dairy Extension Specialists early in the spring of 1927 to get acquainted with the other workers of Extension to learn their problems and get the results of better methods for carrying on of our own work.

4. The report of Bull Associations Committee which is now called "Better Sires" Committee was made by Stanley Brownell of Pennsylvania for Mr. Geo. Gurbach of Michigan.

It was recommended that the terms "Grade Cow," "Proven Sire," and "Bull Association" be defined by a committee. There should be a coördination of the Better Sire work with other states. Now, every state works independent of other states. The committee recommends the Extension Section of the American Dairy Science Association request that the Bureau of Dairy Industry, U. S. D. A. assist by furnishing the necessary field force to coördinate "Better Sires" work between states. They also ask that the A. D. S. A. request the Bureau of Dairy Industry to use their influence to get data on the number and percentages of purebred sires of the dairy breeds by counties in the various states. It was suggested that the registered sires go free from taxation while the grade and scrub sires should be taxed to the limit. A workable plan for the certification of sires of proven merit may be formulated during the next year.

5. The Calf Club Committee report was made by Nevels Pearson of Michigan for T. A. Erickson of Minnesota.

The committee recommends that the club members' individual records and stories be considered in making awards at fairs. The personal effort made by the boy or girl should count in making awards. Most states have their club work on a three year basis. This should encourage the boy or girl to take a permanent interest in the work. Further support is needed to encourage dairy stock judging contests, showmanship, selection of calves and better methods of growing out of calves. The Committee recommends the selection of more heifer calves because often the bull becomes unruly and hard for the child to manage after the first year. The aim of the committee is to develop breeders of good purebred stock. The committee is asking for assistance to curb the high abnormal prices asked for calves for calf club work. We need the purebred breeders coöperation if the boys and girls are to become successful breeders of good dairy stock.

6. The Dairy Manufacturers Committee did not send a representative or hand in a report.

A motion was passed to the effect that the Program Committee arrange that the Production and Extension Sections do not hold their meetings at the same hours.

The Nomination Committee re-appointed the officers of this year to serve for the coming year, namely:

A. J. Cramer, Chairman, Madison, Wisconsin.
A. C. Baltzer, Vice Chairman, East Lansing, Michigan.
C. R. Gearhart, Secretary, State College, Pennsylvania.

(Signed) C. R. GEARHART, *Secretary.*

OFFICIAL TESTING SECTION

The meeting of Section IV, Official Testing Section of the American Dairy Science Association was held Thursday, October 7, 1926, at the Book-Cadillac Hotel, Detroit, Michigan.

Meeting was called to order by Chairman C. E. Wylie.

Minutes of previous meeting were read by secretary W. E. Peterson and approved. Mr. M. H. Campbell, chairman of the Breeds Relation Committee read report for that committee. Report was accepted. The most important phases of this report are:

1. Addition to official test rules that supervisor and milker refrain from talking during milking.
2. Addition to official test rules that "The supervisor shall run a blank test on all the sulphuric acid, consisting of 17.6 cc. acid and sufficient water to fill the bottle."

3. A resolution to all breed associations requesting them that the revised rules of the American Dairy Science Association, governing official testing be published in the different breed association handbooks.

Mr. C. T. Conklin, secretary of the Ayrshire Breeders' Association reported on the Ayrshire Herd Test Plan. He reported general satisfaction with the present plan and that it had greatly stimulated interest in testing.

Mr. P. S. Williams of Pennsylvania presented a paper on supervising the Herd Test Plan.

Dr. C. H. Eckles of Minnesota presented a paper on The History and Trend of Official Testing.

Mr. R. T. Harris, of Wisconsin, chairman of the Investigations Committee, reported for that committee. The report was adopted as read.

Little work has been undertaken by this committee during the past year.

Darnell of the Texas station reports work still in progress in a study involving the effects of feeding cottonseed meal to dairy cows on official test. More facilities are to be available another year so this study may be continued on a larger scale and valuable results are anticipated.

Petersen of the Minnesota station reports further on the possibility of adulterating ordinary commercial sulphuric acid so as to increase the reading of the Babcock test in which such acid is used. He has been able to secure an acid emulsion that is stable and that will increase the Babcock reading when used in making the test. An increased reading as high as 0.8 per cent is noted. The emulsion is stable and the appearance and action of the treated acid normal. As indicated last year such adulteration can be easily detected by running blank tests using water instead of milk and completing the test in the usual manner.

Harris of the Wisconsin station reports on a trial to determine the practical application of a herd test plan adaptable to all breeds and reduced to the simplest possible terms. After eighteen months he finds that (*a*) records made are reliable, (*b*) some low producers are eliminated, (*c*) good sires located, (*d*) members well satisfied with results and (*e*) test supervisors interested and regard plan with favor. The plan, known locally as the Herd Improvement Record, is based upon the standard cow testing association methods and provides for retests, inspections, checking for accuracy and permanent filing of records. Certificates are issued to herds with average above 300 pounds butterfat in the year, but none to individual cows. Offered as a supplement rather than a substitute for A. R. work. To date about 50 herds entered with 1000 cows, including Brown Swiss, Guernsey, Holstein and Jersey breeders.

Breed representatives were called on for remarks. Lynn Copeland responded for the American Jersey Cattle Club, reporting a marked increase in testing after the adoption of the one-day test.

Mr. Harris of Wisconsin reported for Mr. Gardner of the Holstein Friesian Association that the one-day test has been made optional to breeders doing Class C testing.

Mr. P. S. Williams, State College Pa., and Mr. J. M. Fuller, Durham, N. H., were elected president and secretary respectively.

Meeting adjourned.

WILLIAM E. PETERSEN, *Secretary.*

OFFICERS OF AMERICAN DAIRY SCIENCE ASSOCIATION, 1927

The mail ballot taken in December, 1926, resulted in the election of the following officers:

President: J. B. Fitch, Manhattan, Kansas

Vice-President: J. M. Sherman, Ithaca, New York

Officers of Eastern Division, A. D. S. A.: Chairman, J. M. Fuller, Durham, New Hampshire; Secretary, S. H. Harvey, College Park, Maryland.

Officers of Western Division: Chairman, F. W. Atkeson, Moscow, Idaho; Secretary, N. C. Jamison, Corvallis, Oregon.

Officers of Southern Division: Chairman, J. S. Moore, Agricultural College, Mississippi; Secretary, A. C. Baer, Stillwater, Oklahoma.

Summer Meeting of the American Dairy Science Association

In accordance with the resolution passed at the last meeting of the Dairy Science Association, the Executive Committee has voted to hold a summer meeting at the Agricultural College, East Lansing, Michigan, June 22, 23, 24, 1927.

The tentative plans for the arrangement of the program are as follows:

First Day

10:30 to 12:00 1:30 to 4:00	Instruction in Dairy Husbandry Program will consist of limited number of papers with ample time for discussion.

Second Day

9:30 to 12:00 1:30 to 4:00	Research Program Program will consist of a series of short papers (limit 10 minutes), each followed by a short discussion period.

Third Day

9:30 to 12:00	Extension Methods and Results
1:30 to 4:00	Section 1, Dairy Manufacturers Joint meeting Section 1, Production; and Section 3, Extension. Program to include subjects of special interest to sections and any matters of business needing attention.

No programs will be scheduled for the evenings, leaving opportunity for special conferences, social and recreational activities.

The success of the proposed program depends upon the response of the membership. The committee asks every member of the association and especially those having administrative charge of dairy departments of colleges to give the matter of program prompt attention. Suggestions are invited regarding the program in general. Members desiring to present papers are requested to send title and author at once. Papers presented on Research Program may be in the nature of an abstract of a paper to be presented for publication later, or may be a report of work in progress or completed and not intended for publication; or a discussion of plans and methods of research.

PROGRAM COMMITTEE
C. C. HAYDEN,
Experiment Station, Wooster, Ohio.
H. A. RUEHE,
University of Illinois, Urbana, Ill.
C. H. ECKLES, CHAIRMAN
University Farm, St. Paul, Minn.

BOOK REVIEW

Professor Victor Arthur Rice of the Animal and Dairy Husbandry Department, Massachusetts Agricultural College is the author of a new text book BREEDING AND IMPROVEMENT OF FARM ANIMALS, published by McGraw Hill Book Company, New York.

This book covers in a thoroughly scientific and yet practical manner such subjects as physical basis of inheritance, sterility, reproductive efficiency, genetics, acquired characters, sex determination, grading and cross breeding, inbreeding, selection of farm animals and fitting for sale. It has been written primarily as a text book for students in animal breeding but is also a valuable reference book for the practical livestock man.

J. H. F.

HISTORY OF DAIRYING, a history of the dairy industry representing over 15 years of research by the author, T. R. Pirtle, is now off the press. The volume contains 645 pages, 203 illustrations, and graphs, and covers the subject in all countries of the world.

An interesting feature of the book is the International Chronological Index of Events dated from 4000 B.C. when cattle and dairying were found in Switzerland, through 1000 A.D. with the first record of ground cheese, supposed to be a new process.

This index contains the main steps in the industry including the first cooperative cheese making, in 1380 in the Balkans; record of foot and mouth disease in 1700 in France. Pasteurization of milk discovered in 1857–1862, in France. The thermometer was first used in dairy work in 1870 in Denmark; the cream separator invented in 1878 in Sweden. Butter fat testers were made by Dr. Gerber in Switzerland and by Dr. Babcock in the United States in 1888 and in 1890, respectively.

The work is designed to be of interest as a text book for students and as a reference to leaders in the industry.

The book is published by Mojonnier Brothers Company, Chicago.

J. H. F.

INTERNATIONAL DAIRY EXHIBIT

An International exhibition of dairy machinery will be held in Berlin, Germany, August 16–20, 1927 in connection with the thirty-sixth meeting of the Association of German Dairy Scientists. Information regarding the prizes offered can be secured from Paul Funke, Chausseestrasse 8, Berlin N. 4, Germany.

A METHOD FOR THE SAPONIFICATION OF BUTTER FAT FOR DETERMINING THE REICHERT MEISSL NUMBER*

GEORGE SPITZER AND W. F. EPPLE

Dairy Department, Purdue University Agricultural Experiment Station, LaFayette, Indiana

In this laboratory for the past fifteen years many thousand Reichert-Meissl determinations have been made. During this period we have used, at different times, one or the other official methods. After numerous trials we confined our work to the saponification of the fat under pressure with alcohol, preferring this method to the other two methods. In spite of its apparent simplicity, it frequently happened that the alcohol was not completely removed, due to the solution and mixture with the soap formed during saponification.

We, therefore, tried to saponify the fat without the use of alcohol. After numerous trials we found that by adding concentrated solution of potassium hydrate directly to the 5 grams of fat, the fat being previously heated to 80° to 100°C., shaking slightly to thoroughly mix the fat and potassium hydrate solution. This mixture was placed in an electric oven for thirty to forty minutes. At the end of this time saponification was complete. The results obtained by this method of saponification were checked against the three official methods with very consistent results.

METHOD

Use a 250-cc. low form Griffin beaker, pyrex glass preferred. Balance beaker, place a 5-gram weight on opposite pan and with a pipette run into beaker approximately 5 grams of melted butter fat, weigh added fat to the third decimal place.

Place the weighed fat in an oven heated to 100°C. until temperature of fat reaches 80° to 100°C., remove and add 3 cc. of

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potassium hydrate solution,[1] by a circular motion mix fat and potash solution. After being mixed in this manner incline beaker to an angle of 45° and rotate. Some of the mixture will spread on side of beaker. Continue this rotation until the mixture thickens by cooling. Place beaker in oven at 100°C. and let it remain for thirty to forty-five minutes to complete saponification. Place beaker in desiccator to cool also to prevent the absorption of CO_2.

To transfer the saponified fat to the distilling flask break the cake of soap into small pieces by means of a glass rod, transfer the broken pieces to flask, the adhering particles on beaker and glass rod are removed by means of previously boiled hot distilled water. This water must be accurately measured. The total volume added should be 130 cc. When soap is dissolved in the distilling flask add 10 cc. of dilute sulphuric acid A. O. A. C. P. 247—23 (d), then proceed as directed by the official method, p. 248—25, 1920.

ADVANTAGES OF THIS METHOD

1. Speed in weighing the fat, weighing is made directly by pouring the fat into the counterpoised beaker. Variations from 5 grams need not exceed + or − 0.1 gram.
2. Rapidity in saponifying the fat and transferring the saponified fat to the distilling flask.
3. No foreign substances such as alcohol or glycerine are used, either of which may effect the results.
4. The contamination with CO_2 is reduced to a minimum.

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[1] The solution of potassium hydrate is that of the A. O. A. C. method, page 247–23 (b), 1920, i.e. 100 grams of KOH to 58 cc. of distilled water and protect from CO_2.

"SYNTHETIC MILK" AS A BASIS FOR RESEARCH*

W. MANSFIELD CLARK

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Some time ago, while attempting to formulate the chief features of the acid-base equilibria of cows' milk, my attention was called to two distressing matters of very considerable importance to milk chemistry and milk technology. In the first place I found in the enormous literature no analysis of milk sufficiently complete and at the same time sufficiently well interpreted for my purpose. One of the several deficiencies lay in the interpretation of the origins of the phosphate appearing in the ash. Without definite and unequivocal knowledge of the distribution of phosphorus between the various organic forms and the phosphate entering the buffer action of milk, there could be no assurance in the treatment of buffer indices. The second matter was the lack of adequate information upon the conduct of even simple systems in which phosphate, calcium and citrate take part simultaneously in acid-base equilibria. Without this the next important step for milk chemistry description of the conduct of such systems in the presence of milk proteins could not even be guessed.

The question of the analytical composition of milk has always been in some confusion because of the well known variability. This has needlessly overshadowed the advantages which could be gained from a complete physical and chemical analysis of one sample made with due consideration for the sources of error and with those types of duplication which would insure reasonable completeness for purposes of interpretation. The question of the conduct of simple systems involving citrate, phosphate and calcium may appear beside the point to one whose attention is focused upon the *variability* in the physical stability of cows' milk, for it may be argued that each milk is, in a sense, a new problem, and the study of variants is of more immediate impor-

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* **Published by permission of the Surgeon General, United States Public Health Service.** **Received for publication December 27, 1926.**

tance to milk technology than is so academic a question as the conduct of a definite simple system remote from a complete milk. With such a point of view I would disagree most vigorously. The very fact of variability, both in chemical composition and in physical stability, precludes simultaneous studies having the completeness that the problem demands. One has only to inspect the existing data to realize that the correlations which it has been possible to make with the equipment and personnel of one laboratory are very limited, and that there lingers about every case the suspicion that undetected changes, as the investigator passes from case to case, may vitiate some of the sweeping conclusions that have been drawn.

Now there should be no fundamental difficulty in establishing a systematic survey of the citrate-calcium-phosphate equilibria mentioned. Much can be done with the *combination* of analytical measurements and hydrogen electrode measurements. Much more can be done if the calcium electrode can be developed. Still more will be done if the limitations in the classic equations for acid-base equilibria are recognized and dissociation constants and solubility products applicable to the environment of milk are determined and used in place of the constants determined for dilute and simple solutions.

In the meantime, it seems to me that some advances can be made with the use of a synthetic milk the gross composition of which can be determined by the ingredients used and the physical properties of which can be determined roughly at least by the manner of preparation.

Synthetic milks have been proposed before, more as curiosities than as a serious basis for experimentation. Porcher and Chevallier (1923) have cited these in their elaborate review of the composition of cows' milk. The fundamental difficulty with those proposed, as with Porcher and Chevallier's choice of terms in which to express the composition of true milk, is the use of specific salts which are assumed to exist as such in milk. In the first place, there is no justification whatever for neglecting the labile equilibria among the ions of the salts mentioned. The specification of definite salts gives an entirely erroneous impression. In the second place, the fact of an irreversible shift in equilibria on the heat-treatment of milk shows definitely that a stable equilibrium in the original milk has not been reached and the attempt to duplicate the conditions of milk by the use of salt mixtures which will tend toward stabilized equilibrium before the final product is formed defeats one of the purposes to which a "synthetic" milk can be put.

Neglecting for the time being the minor constituents of true milk and the so-called excretory products found therein, we may arbitrarily assume the composition listed in table 1.

TABLE 1

Assumed composition of milk

	GRAMS PER LITER	EQUIVALENTS PER LITER	MOLS PER LITER
K_2O	1 80	0 0382	
Na_2O	0 72	0 0232	
CaO	1 78	0 0635	
MgO	0 30	0.0148	
Total		0 1397	
P_2O_5	1 50		0 0211(H_3PO_4)
Citric	2 00		0 0104
Cl	1 00		0 0282
SO_3	0.11		0 0014
Total CO_2			0 0050
Casein	28 0		
Albumin	7.2		
Other protein	0.2		

There are innumerable ways in which a system of the *composition* cited can be constructed. However, there is at least one very definite limitation upon practical ways and means. This is the excessively large proportion of calcium which must be handled with sulfate, citrate and phosphate. The very low solubility products for certain salts of calcium and these ions will result in premature precipitation and a final product not remotely resembling milk. Therefore, the problem is largely one of choosing that combination of salts and of choosing an order of mixing the solutions which may be presumed to furnish the desired product. Whether or not this product represents the conditions of true milk remains to be seen. But it is the possibility of *controlled* variation in *composition* and in *physical state* that should make such a product a valuable material of research.

Of several possible schemes the following seems promising.

In its use the albumin and other proteins not casein were not available and were replaced by the equivalent of casein.

	Mols			
Solution 1	0 0074	MgO	0 298 grams	Dissolve with warming and dilute to 100 cc.
	0 0211	KH_2PO_4	0 873 grams	
	0 0104	Citric acid. H_2O	2 185 grams	
Solution 2	0 0014	$CaCO_3$	0 140 grams	Dissolve cold. Dilute to 50 cc. and use while fresh
	0 0014	H_2SO_4	28 cc. N/10 H_2SO_4	
Solution 3	0 0141	$CaCl_2$	1 565 grams or preferably the equivalent of an analyzed solution. Dilute to 50 cc.	
Solution 4	0 0171	KOH	17 1 cc. N/1 KOH	Dilute to 50 cc.
	0 0232	NaOH	23 2 cc. N/1 NaOH	

Solution 5 (for 200 cc. "synthetic milk" in which casein represents total protein)

7.0 grams casein
10 0 grams lactose } in 130 cc. N/20 $Ca(OH)_2$ solution

To prepare a lime-water as strong as N/20, thoroughly saturate the solution while it is cooled with surrounding ice. Add this slowly to the casein while the casein is being ground in a deep glass mortar. Finally stir with a motor-driven stirrer.

Add 10 cc. of solution 4. When the solution is free from suspended material add 10 grams lactose. As soon as this is dissolved add at once 10 cc. of solution 3 and 10 cc. of solution 2. Now set the motor at high speed and to the rapidly stirring solution add very slowly from the *fine* tip of a pipette 20 cc. of solution 1. When this addition is complete dilute the "milk" to 200 cc.

"Milks" prepared in this manner have the appearance of a very "blue" skim milk.

A sample in which the KOH-NaOH mixture was replaced by

NaOH and in which the casein employed was an unanalyzed sample of Harris casein "Nach Hammarsten" yielded the following data.

It was stable in the sense that no settling of the solid phase occurred when centrifuged one-half hour at 2500 r.p.m. in a large centrifuge (constants of centrifuge not determined). When sterilized by heat in a sealed tube a slight precipitation was observed after eight months. Analysis for N showed only 3 per cent casein indicating a high moisture content in the original

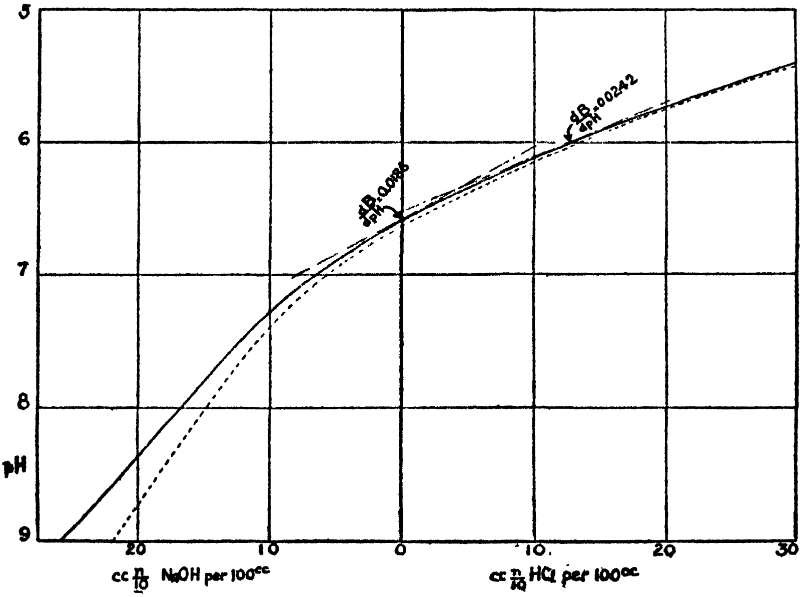

Fig. 1

casein and some little loss by adherence to the walls of the preparation vessel of a few grains of the coarse preparation. Total solids by customary procedure 8.44 per cent—representative value for solids not fat in real milk, is about 9. Ash by customary procedure 0.65—representative value for real milk 0.7. By reason of the absence of the minor constituents of milk the sample should show not only appreciably lower percentage composition but also an appreciably higher melting point. Two samples gave −0.521°C. as compared with −0.55°C. for true milk.

This synthetic milk formed rapidly with rennet a typical curd. Heating to boiling temperature in a test tube produced a more markedly milky appearance. The rennet action was then delayed.

No effort was made to equilibrate the solution with respect to normal CO_2 content. This can be done subsequent to the preparation. Measured as prepared and with a hydrogen electrode at 30°C. it gave an initial pH of 6.66. A representative value for milk is 6.6 ± 0.1.

The titration curve is shown in figure 1 by the dotted line. The good agreement between this titration curve and the titration curve of a real milk shown in figure 1 by full curve should not deceive the reader. In neither case are details of composition accurately known. Therefore it is only the gross aspects of the agreement which are worthy of present discussion.

Regarding the titration curve of true milk shown in figure 1 as typical, we find by graphic methods $\frac{dB}{dpH} = 0.0186$ at pH 6.6 and $\frac{dB}{dpH} = 0.0242$ at pH 6.0. Practically the same values apply to the "synthetic milk."

The "synthetic milk" described above should provide a material with which systematic studies can proceed. It is necessary, however, to give this warning. It will probably be found that the inclusion and the omission of the protein will furnish two radically different buffer values for the other components according to whether hydrogen electrode measurements are made on the system at equilibrium or before sufficient time for true equilibrium has elapsed. Compare, for instance, the curves for phosphate-Ca systems as described on the one hand by Davis, Oakes and Salisbury (1923) and, on the other hand, by Holt, LaMer and Chown (1925). The proteins must be regarded as not only contributing to the acid-base equilibria directly but as conditioning the state of solid phases. The latter effect will probably have to be duplicated by non-electrolytic colloids when the ionizable proteins are withdrawn.

The order in which components are mixed and the rate of mixing will doubtless be found to influence the colloidal state of the product. In this connection it may be said that it seems highly improbable that certain of the dispersed but undoubtedly solid phases in which certain of the salts occur could be secreted as such by the mammary gland. Therefore, it is not too much to suggest that ingenious modifications of the rather crude scheme outlined above might lead to knowledge of components which it would be *possible* for the gland to secrete independently.

Of immediate importance is the use of such a controlled product in solving quantitatively the problem of the state of calcium and phosphate in milk. This is a matter of considerable importance not only to the technology of milk but to public health. We are informed that a very large proportion of our people show, in bone structure, evidences of incipient rickets and that the margin of safety in the assimilation of calcium and phosphate is extremely narrow. Improvement in the modification of cows' milk for infant feeding can hardly proceed with intelligence until the problem mentioned above is solved and solved in the exact and quantitative language of equilibrium data.

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Holt, LaMer and Chown 1925 Jour. Biol. Chem., xlvi, 509.
Porcher and Chevallier 1923 Le Lait, iii, 97, 188, 289.

THE RÔLE OF GELATIN IN ICE CREAM*

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The writers assume in this paper that a certain amount of viscosity is desirable. If it is desirable, what ingredient or group of ingredients is responsible for the viscosity as determined by the MacMichael Viscosimeter? Dahlberg of the New York Experiment Station, and others as well as this station, found that butterfat exerted some influence upon the viscosity of the mix. The milk-solids-not-fat exert more influence than does the butterfat but none of the dairy products exert the influence upon viscosity that does gelatin. As an illustration an ice cream mix containing no gelatin had an initial viscosity of 64.9 centipoises upon cooling to 20°C. Five-tenths per cent gelatin was added to part of the same mix and immediately cooled to 20°C. and the initial viscosity was 237 centipoises. An ice cream mix containing no gelatin will increase its viscosity. It is not unusual to obtain an increase of 500 per cent or greater in apparent viscosity due to the addition of gelatin.

Since gelatin seems to be the most important ingredient in obtaining viscosity, it will be the purpose of this paper to discuss the partial rôle of gelatin in an ice cream mix.

Gelatin is a protein and may be defined as the most typical of reversible colloids. Due to its chemical and physical properties, it is an excellent stabilizer of emulsions. The importance of stabilizing an ice cream mix is recognized, and it is being given a great deal of consideration.

There are two types of viscosity in an ice cream mix. One may be defined as real viscosity which is found in all solutions of crystalloids and colloids alike which have no connection with colloidal behavior. The other may be called apparent viscosity

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† Some of the data for this article were taken from the work of Mr. Milner presented as partial fulfillment of the requirements for his M.S. degree.

which is viscosity due to the swelling of submicroscopic solid particles in a solution. The apparent or colloidal viscosity has a greater order of magnitude than real viscosity.

The difference in the magnitude of casein and gelatin is due (1) to the fact that the gelatin possesses greater mechanism for increasing its relative volume in solution. The submicroscopic particles of gelatin or micellae occlude large amounts of water whereby the relative volume occupied by the gelatin is increased, these microscopic particles being the forerunner of a continuous gel to which the gelatin solution has a tendency to set.

There undoubtedly is a colloidal structure built up in an ice cream mix which has varying degrees of stability. This fact is borne out by Bogue (2) and others.

TABLE 1

Effect of quality of gelatin on viscosity

Number 30 certified wire

NUMBER	VISCOSITY (POISES)	
	Twenty-four hours	Forty-eight hours
1	2.60	9.36
2	9.36	15.08
3	13.52	26.00
4	28.60	Set*

* Too heavy to run.

The viscosity of a crystalloid solution is dependent only upon concentrations and temperature while in the case of colloidal viscosity we have quality, concentration, temperature, time and agitation. In the case of an ice cream mix the concentration is largely dependent upon the quality of the gelatin used.

Bogue states that the gel consistency is proportional to the undergrade protein present in the gelatin.

The quality of the gelatin has much to do with the viscosity (3) as shown by table 1.

The gelatins used above represented four grades and had a large range of gel strength from poor to excellent. The composition of the mixes in all cases was the same—11.00 fat and 36.84 total solids, 0.5 per cent gelatin in all four mixes.

Hatchesk states that the formation of a gel structure will vary with the brand and grade of gelatin. Less than 0.25 per cent will not set even at 0°C.

EFFECT OF AMOUNT OF GELATIN ON VISCOSITY

Our work on gelatin concentration bears out the work of Downey and others in proving that with each increase in amount of gelatin there was an increase in viscosity. The increase in viscosity was not nearly so great in smaller quantities (less than 0.4 per cent) as above. However, this depends largely upon the grade of gelatin used. (See table 2.)

TABLE 2

Effect of varying amounts of gelatin

34 wire

TIME AGED	AMOUNT OF GELATIN	VISCOSITY CENTIPOISE
hours	*per cent*	
24	0.2	64.6
24	0.3	102
24	0.4	428
24	0.5	1224
24	0.6	2892

This particular mix contained 10.78 per cent fat, 36.79 per cent total solids, to which gelatin was added in varying amounts (0.2 to 0.6 per cent).

The gelatin used in this mix was one of the better grades of gelatin.

The temperature of the mix during the aging period is important if the maximum amount of viscosity (all other factors being constant) is to remain in the finished ice cream. The colloidal structure derived from gelatin is very unstable under certain conditions. Von Schraeder (8) points out that the viscosity of a gelatin solution increases 0.1 per cent in sixty minutes, while by lowering the temperature to 24°C. there is an increase in sixty minutes of 1.5 per cent, and at 21°C. there is a 750 per cent increase in sixty minutes. As the temperature decreases the threads lengthen and at the same time the water adsorbing power increases. This accounts for the rapid increase in viscosity with decreased temperature.

The increase in rigidity of the gelatin structure will depend upon the amount of the free solvent in the interfaces and the amount of solvent that has been taken up in the hydrated and imbibed condition.

The time required to age a mix properly is not the same for all mixes. If a poor grade of gelatin is used more time must be allowed for the gelatin to hydrate and the viscosity to develop than with a good grade of gelatin.

If viscosity is such an important factor and gelatin is the most important factor in obtaining viscosity, what, then, are some of the characteristics of gelatin that tend to stabilize viscosity?

Some authorities state that a good gelatin should make up a clear solution. Our study of gelatin does not indicate that color bears any great definite relation to jell strength, solubility, rate of setting or odor.

We find gelatins on the market varying in pH that make up clear solutions in water and by changing their pH they become turbid upon approaching the isoelectric point. We may take a turbid gelatin and make it clear by a change in pH away from the isoelectric point. By altering the pH away from the isoelectric point, we weaken the gel strength and viscosity. This is accounted for by Bogue (7) who states "That the greatest opacity results from large aggregates of less swollen particles. This maximum of opacity occurs at the isoelectric point. Any decrease in size of the aggregates or increase in hydration results in greater clarity or transparency of the solution."

This means that almost any gelatin may be made clear or turbid by altering its pH away from or toward its isoelectric point.

Table 3 illustrates the effect of the pH on the clarity of the gelatin solution.

We have found at least one exception which was undoubtedly due to the partial removal of the turbidity by the process of manufacture which does not necessarily improve the quality of the gelatin for the manufacture of ice cream, though it does improve its general appearance in a water solution which is the way we have come to know gelatins.

The gel strength is influenced markedly by the pH of the gelatin as shown by the inversion test. Bogue (9) points out that the maximum gel consistency is at the pH of 4.0 to 4.5. In our work we used 1.5 per cent gelatin in water solution. The writers found that the gel consistency increased as the gelatin solution approached the isoelectric point. A weak gelatin may be strengthened if it has, for illustration, a pH of 7.0 or 3.0 by changing it to approximately 4.7. This does not mean that it would be possible to take a poor grade gelatin and by changing the pH make a high grade gel, although it may be improved somewhat. The odor of gelatin in water solution warmed to approximately 140° to 160°F. will indicate somewhat the grade.

TABLE 3

Effect of the pH on the clarity of the gelatin solution

SAMPLE NUMBER	pH	COLOR
1	7.0	Clear
1	4.7	Turbid
1	3.0	Clear
4	5.3	Turbid (slightly)
4	4 7	Turbid
4	3.0	Clear
2	6.6	Clear
2	4.7	Turbid
2	3 0	Clear

Some gelatins were found to be apparently free of liquefying and putrefactive organisms but the pH was so high that they would not grow, and by changing the pH to approximately that of an ice cream mix these same gelatins showed the presence of large numbers. Some gelatins are sold to the trade with a pH so that those undesirable types would develop upon incubation in a water solution, while others would not.

Since the strongest gel is obtained when the gel is at a pH of approximately 4.7 and an ice cream mix under factory conditions is approximately 6.30 (we realize that this figure varies slightly, depending upon source and quality of products used) the question arises whether the large reservoir of salts in the mix is sufficient to change the pH of the gelatin without materially changing the pH of the ice cream mix. If this is so, then gelatin should be purchased upon a little different basis than in the past.

TABLE 4

Effect of pH of gelatin upon pH of mix

MIX NUMBER	SAMPLE	pH OF GELATIN	pH OF MIX
A	1	7 6	6 27
A	2	3 8	6 23
A	3	3 8	6 17
A	4	4 0	6 18
A	5	No gelatin	6 24
B	6	4 7	6.42
B	7	3 0	6 21
B	8	6 3	6 25
B	9	8 0	6 30
B	10	No gelatin	6 31
C	11	4 2	6 33
C	12	7 0	6 37
C	13	3 8	6.23
C	14	5 8	6 26
C	15	No gelatin	6 30
D	16	3 8	6 27
D	17	7 0	6 33
D	18	4 0	6.26
D	19	7 6	6 28
D	20	No gelatin	6 29

As would be expected, the usual range in the pH value of gelatin affects but little the pH of an ice cream mix. The effect, if any, is of no commercial importance.

By referring to table 4, we conclude that the gelatin, regardless of its pH, changes to that of the ice cream mix. Undoubtedly there would be present in the average ice cream mix sufficient salts to change the pH of the gelatin to that of the mix. In table 4, each mix was divided into five parts and 0.5 per cent gelatin added with varying pH values to four of them, and to the fifth, no gelatin was added. All of the above readings represent four or more pH determinations. The variations in a mix with different gelatins, while excessive in some types of solutions, are not considered excessive by the authors, due to the complexity of an ice cream mix.

The pH determinations were made by use of the Type "K" Potentiometer using a Clark cell, the sample being bathed with a flow of hydrogen generated by hydrolysis. Uniform technique was followed in all samples. The pH of gelatins can easily and accurately be determined by the colorimetric method as described by Brown (4).

Many authorities are of the opinion that good gelatin aids in preventing the formation of ice crystals. It has been the writers' observation that the gold number is of little value in the evaluation of the quality of gelatin. It has been our experience that ice cream containing no gelatin has a greater tendency to form ice crystals. The concentration and quality of the gelatin has much to do with this phenomena. It was partially explained by Menz (5) when he showed that the protective action of gelatin is increased as the concentration is decreased. The work of Menz was corroborated by Elliott and Sheppard (6). Their conclusions were that the protective action is dependent upon the concentration of amicrous present in the solution.

SUMMARY

1. Gelatin seems to be the most important ingredient in obtaining viscosity as determined by the MacMichael.
2. Two types of viscosities are present in an ice cream mix which may be defined as real and apparent. The latter is very unstable under certain conditions.
3. The quality of gelatin is largely responsible for the amount of viscosity that may develop during aging.
4. The color of the gelatin and water solution does not necessarily indicate the quality of the gelatin.
5. The gel strength of the gelatin is influenced markedly by the pH.
6. Regardless of the initial pH of the gelatin, it assumes the pH of the ice cream mix probably due to the reservoir of salts and the small quantity of gelatin added (0.3 of 0.5 of 1 per cent).

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(7) Bogue: Technology and Chemistry of Gelatin and Glue, p. 156.
(8) Von Schraeder: Zie Physik Chem., vol. 45 (1903), p. 75.
(9) Bogue: Technology and Chemistry of Gelatin and Glue, p. 154.

STUDIES ON YEASTS IN DAIRY PRODUCTS

III. THE PINK YEASTS COMMON IN MILK AND CREAM*

W. A. CORDES AND B. W. HAMMER

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INTRODUCTION

The yeasts forming pink colonies are very conspicuous among the organisms isolated from dairy products. While there is considerable variation in the color produced, this does not ordinarily extend to the point of questionable color production; occasionally a subsurface colony or even a young surface colony on certain media may fail to show a definite pink color, especially on heavily seeded plates, but usually color is produced in such amounts that it is readily recognized whether the colony develops on a plate or on some dairy product. Except for the pink yeasts, color production among the yeasts present in dairy products is unusual; in the examination of many hundreds of plates poured from dairy products for the isolation of yeasts, colonies producing pronounced colors other than pink have been encountered only a very few times.

A study was made of a considerable number of pink yeasts isolated from dairy products and the results are herein reported. From the data it appears that there is a common type but that certain cultures show variations from this; accordingly the group is divided into types and each characterized.

DISTRIBUTION OF THE PINK YEASTS IN DAIRY PRODUCTS

The pink yeasts have been repeatedly isolated at the Iowa Agricultural Experiment Station from a wide variety of dairy products. Sweet cream coming in from the college cream routes or delivered by farmers often contains a few hundred and occa-

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sionally a few thousand per cubic centimeter. Sour cream nearly always contains these organisms and sometimes they are present in considerable numbers. Like many of the other yeasts, the pink forms are capable of withstanding considerable amounts of acid and accordingly they sometimes appear on the surface of sour milk or cream or on soft cheese as distinct colonies that are usually round, smooth-edged and considerably raised. In some instances they occur singly or at least in small numbers while in others there may be a considerable number of colonies irregularly arranged or in a very distinct row or series of rows; the rows of colonies are undoubtedly due to a breaking up of a clump in quite the way a row of colonies occurs in an agar plate or, in the case of soft cheese, to contamination from a paddle used in handling the product.

Although pink yeasts have been repeatedly isolated from butter, colonies have never been observed on this product. The pink yeasts are undoubtedly present in butter because of their distribution about the creameries and their ability to withstand an unfavorable environment. While it is possible that they might be capable of forming colonies in butter under certain conditions these are apparently not usually provided.

GENERAL CHARACTERS OF THE PINK YEASTS

The pink yeasts isolated from dairy products have certain characteristics aside from the color produced in colonies on plates or other materials.

Changes are produced in milk rather slowly even in the presence of large amounts of growth as evidenced by the mass of pink color in a ring at the surface of the milk and in the sediment at the bottom. The production of a large amount of growth with comparatively little change in the milk in which this growth occurs is rather usual among the yeasts found in dairy products and is well illustrated by the pink type. After a considerable time milk in which the pink yeasts are growing coagulates and accompanying this there is more or less reduction of the litmus, particularly at the bottom of the tubes, while the color remaining indicates that the reaction tends toward alkaline; certain cultures then digest the milk, and this digestion may eventually be very complete.

The temperature requirements of the pink yeasts are also of interest. These organisms in general do better at 21°C. than they do at 37°C., and some fail to grow at all at the latter temperature. Other groups of yeasts also seem to have their growth seriously interfered with at temperatures that are unusually favorable for many types of bacteria so that this relationship is not characteristic of the pink yeasts alone.

The colonies produced are usually approximately round on the surface of the plating medium and round or elliptical beneath the surface. Both the surface and subsurface colonies are quite smooth-edged and in the subsurface colonies there is no development of fingers or mycelium-like structures that project out into the medium and suggest mold colonies as is the case with many yeasts that give a typical yeast-like surface growth. Sometimes in old plates some of the colonies develop fissures from which cells grow out and form a structure suggesting a new colony, but this secondary growth is again smooth-edged; the breaks in the edge of the old colonies are more likely to occur in subsurface colonies than in surface colonies and are most frequent along the sides of elliptical colonies.

CULTURES STUDIED

Approximately 90 cultures of pink yeasts were studied in more or less detail. These were largely secured from milk, cream, soft cheese and butter obtained in the vicinity of Ames; many of the cultures were picked from plates poured for the purpose of total yeast and mold counts. For the most part only one culture was selected from a plate so that the cultures studied came from a considerable number of samples of various materials. A transfer of a culture isolated from oysters by Hunter[1] was supplied by Dr. Thom and included in the cultures investigated.

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[1] A pink yeast causing spoilage in oysters. U. S. Dept. Agr. Bul. 819, March, 1920.

VARIATIONS IN THE CULTURES STUDIED

A very large per cent of the pink yeasts studied very evidently belong to the same species which is referred to as the common type. Variations in the intensity of the color produced, in the rate of growth at different temperatures, etc. were noted among these cultures, as would be anticipated, but undoubtedly these simply represent variations within the species and are of little significance from the standpoint of classification except to help establish the variations to be expected. However more pronounced variations which follow through several characters were also noted with a few cultures. Two groups of these pronounced variations were encountered and the organisms showing them were considered to constitute two types distinct from the common type; these variations are given in detail following the description of the common type of pink yeast.

DESIGNATION OF THE COMMON PINK YEAST

The common type of pink yeast studied apparently resembles very closely the organism referred to as Torula glutinis or Cryptococcus glutinis. However, Guilliermond[2] has pointed out that, "Torula glutinis does not possess a very characteristic appearance and a series of related yeasts have been described under this name"; a consideration of the literature shows that this statement is amply justified. Golden and Ferris[3] in discussing T. glutinis state that, "An error seems to have crept into the description of this species in regard to its size, the other characters being practically the same in all the descriptions."

It should perhaps be recognized that in the determination of certain characters there are likely to be discrepancies in the results reported by different laboratories due to variations in the methods even when the same cultures are used and it is possible that these variations would be even greater if different cultures of what should be considered as the same species were employed. The pink yeasts, although giving a rapid growth, bring about few bio-chemical changes and these only very slowly, so that the period over which the observations are continued may be an important point. Hunter reported, "No coagulation nor peptonization" of litmus milk for the yeast he isolated from oysters, while the culture from Hunter's isolations that was included with those discussed herein gave, after a rather long period, both coagulation and digestion.

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[2] The Yeasts. Translation by Tanner, p. 318.

[3] Red Yeasts. Bot. Gaz., 1898, xxv, p. 45.

It seems advisable to consider the common yeast that was found in dairy products examined as Torula glutinis and to depend on the use of this name and additional investigations of the pink yeasts to develop a definite meaning and significance for it.

DESCRIPTION OF THE COMMON PINK YEAST—TORULA GLUTINIS

Morphology

Form. The cells were oval. Although spherical appearing cells were frequently noted these were presumed to be oval cells seen from an end. Elongated, thread-like cells were not observed, even in subsurface colonies.

Size. The cells varied greatly in size with the length usually ranging from 2.5 to 4.0 microns and the width from 1.5 to 2.5 microns.

Arrangement. Most of the cells were single, although budding was common. Buds were most numerous in young cultures but were also observed in cultures several weeks old. Only one bud was found attached to each mother cell and this always appeared at the smaller end. It was unusual to find cells attached unless one was somewhat smaller than the other.

Staining reaction. The cells stained readily. Most of the cells in young cultures from both liquid and solid media were gram positive while a few were gram negative. Older cultures showed a greater proportion of the cells gram negative.

Spore formation. Spore formation was never observed.

Cultural characteristics

Whey agar slope. At room temperature growth was quite rapid; it was usually distinct in twenty-four hours and increased rapidly for several days. The growth was pink, filiform, raised, glistening, and non-viscous. Certain variations occurred in the rate of growth and in the intensity of the color produced.

Beef infusion agar slope. Growth was quite like that on whey agar in both character and amount.

Beef extract agar slope. Growth was quite like that on whey agar in both character and amount.

Whey agar stab. At room temperature growth, after twenty-four hours, was distinct, especially at the surface, and increased rapidly. The surface growth was pink, raised, smooth-edged, glistening and non-viscous. Along the stab the growth decreased rapidly from the surface and was filiform in character, with the color less pronounced than at the surface.

Whey agar plate colony. At room temperature colonies were frequently evident after twenty-four hours while after forty-eight hours the surface colonies were often up to 1 mm. in diameter and the sursurface colonies about one-third of this. Growth continued rather rapidly. The surface colonies eventually became several millimeters in diameter and were round, pink, considerably raised, smooth-edged, glistening and non-viscous, while the subsurface colonies were round to ellipsoid, somewhat pink, and smooth-edged. No filaments or thread-like structures were observed.

Whey gelatin stab. Growth was essentially like that in a whey agar stab, with no evidence of liquefaction even after several weeks.

Bouillons. Plain bouillon and bouillons to which additions had been made showed a turbidity and frequently some pink sediment after twenty-four hours at room temperature. Growth increased and a heavy pink ring was often formed at the surface. Eventually the turbidity cleared. Certain sugars, particularly the monoses, seemed to favor growth to a certain extent.

Potato. At room temperature growth was evident after twenty-four hours and increased rapidly; it was raised, smooth-edged and decidedly pink. In very old cultures in which the potato had dried up the color seemed to be brown rather than pink.

Dunham's solution. Growth was evident as a fine turbidity after twenty-four hours at room temperature. As the growth continued a fine sediment developed and as this increased it became quite pink. The turbidity eventually cleared. Frequently there was a tendency to an increased growth at the surface but no distinct ring or pellicle formed.

Uschinsky's solution. Growth was comparatively slow but was evident as a distinct sediment after three days at room temperature. The sediment increased in amount as the cultures aged and became pink in color.

Litmus milk. At room temperature growth was evident in two or three days as a pink sediment and a beginning ring at the surface, the ring often appearing rather granular. A slight reduction of the litmus at the bottom of the tube was sometimes observed. As the cultures aged the ring and sediment increased in amount. Eventually the milk coagulated but this usually required several weeks; the coagulation commonly began at the bottom. Most of the coagulated cultures showed considerable reduction in the lower portion of the tubes with a pronounced blue color at the surface.

Soured milk. Growth was rapid on the surface of soured milk at room temperature. The growth either in stabs or on slopes resembled the growth on whey agar in character and amount.

Bio-chemical features

Gas production. Gas was never observed either in milk or in bouillons to which various additions had been made.

Oxygen relation. The general character of the growth in various cultures indicated the preference of the organism for an abundant air supply.

Reaction change. The organisms apparently produced changes in reaction only slowly. Milk became alkaline after considerable periods. Neutral bouillons to which mannitol, salicin or inulin had been added, were alkaline after seven days at room temperature. Bouillons with glucose or levulose gave acidities from 0.4 to 0.9 per cent N/1 acid while bouillons with galactose, maltose, sucrose or lactose showed still lower acidities.

ADDITIONAL TYPES OF PINK YEASTS

The types of pink yeasts which show pronounced variations that follow through several characters are as follows:

Type A. Torula rubicunda. Several cultures of this type were secured from a number of different sources. The first culture was obtained from a sample of well water. The culture isolated by Hunter was also grouped here. Later a number of cultures isolated in a dairy barn were included. The variations distinguishing this type from the common type are:

1. The pink color produced was very intense. This was true with all the different media used and involved both the surface growth on solid media and the sediment in liquid media.

2. The cells were larger than those of the cultures of the common type, and this greater size occurred with various media. The width of the cells usually varied from 2.0 to 3.3 microns and the length from 2.5 to 6.0 microns.

3. In very old milk cultures there was commonly a digestion of the casein. This digestion never appeared until the cultures were several weeks old and sometimes it failed to appear at all, due possibly to the drying up of the milk. Digestion was never noted in the cultures of T. glutinis.

Type B. Torula paraglutinis. Only two cultures of this type were found and these came from the same sample of cream. The variations distinguishing this type from the common type are:

1. With all the different media used growth was quite slow. On whey agar at room temperature about three days were required for the colonies to be evident while with the usual cultures colonies were evident in one day and in three days were often up to 2 mm. in diameter.

2. In liquid media the growth was more flocculent than turbid, while turbidity was common with T. glutinis in liquid media.

3. With all media the color tended to be slight, and with certain sediments developed in liquid media the pink color was so slight as to be scarcely detectable.

4. On agars the growth tended to be viscous.

5. At 30°C. growth was negative or questionable. With the usual cultures growth was very rapid at this temperature.

DESIGNATION OF THE PINK YEASTS SHOWING VARIATIONS FROM THE COMMON TYPE

Although Type A and Type B show many of the general characters of the common type of pink yeasts they seem to constitute distinct species for which descriptions could not be found in the literature. With both of these types the number of cultures studied is too small to make possible a description that shows the variations to be expected but because of their relationship to the common type of pink yeast it seemed advisable to designate them tentatively. The name Torula rubicunda is accordingly proposed for Type A and Torula paraglutinis for Type B.

SUMMARY

Approximately 90 cultures of pink yeasts were isolated and studied in more or less detail. These came largely from milk, cream, soft cheese and butter obtained in the vicinity of Ames. It was found that most of the cultures belonged to a common type, which is considered to be Torula glutinis, although it is smaller than the dimensions given in most of the descriptions of this organism. Two other types were encountered in small numbers and named Torula rubicunda and Torula paraglutinis to make possible their distinction from the common type. A description of Torula glutinis is given together with a list of the variations from this type shown by Torula rubicunda and by Torula paraglutinis.

SEPARATION OF CANE SUGAR FROM WATER ICE*

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Makers of ice-cream products occasionally find a water ice in which the cane sugar has crystallized, making the product hard, chalky in appearance, and unpalatable. The researches described in this paper may help those who have experienced such a difficulty, since the conditions governing the crystallization of sucrose from frozen solutions have been established with considerable exactness.

In considering this problem, the solubility, temperature, and freezing-point relationships of cane-sugar solutions of varying concentrations should be established if possible. Those temperatures incident to normal ice cream work are, of course, of primary interest.

Upon the cooling of an unsaturated dilute solution to the proper point, ice separates from it. This separation concentrates the sugar in the unfrozen part, and as the temperature lowers this concentration increases. By plotting temperatures and concentrations incident to these temperatures, the freezing-point curve is obtained. Similarly, if a highly concentrated solution is cooled, its saturation point is reached, after which, under suitable conditions, sugar crystals are thrown out of solution. This process lessens the concentration of the sugar in the water, and increasingly lower temperatures are necessary to force out further crystals. These data, plotted in a manner similar to the freezing point data, yield the solubility curve. These curves approach each other, and at their point of coincidence is found a solution saturated to sugar and in equilibrium with ice. Under ideal conditions further abstraction of heat from this mass does not lower the temperature but brings about the separation of both sugar and ice until the mass has become solid, after which the temperature is of course lowered with further removal of heat.

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This point of complete solidification is known as the eutectic point. It is at once evident that if a sugar solution is frozen of a concentration less than that represented by the eutectic, sugar can not be separated from it until this eutectic temperature is reached, because the solution will not until then become saturated with sugar. This means, then, that if water ice is not brought to a temperature lower than the eutectic for cane sugar, the sugar can not separate. In other words, if the ice is not stored at too low a temperature this difficulty can not be experienced. The purpose of the work outlined in this paper is to determine the eutectic temperatures for cane sugar.

Guthrie (1) in a paper published about 30 years ago states that this eutectic temperature is about −8.5°C. (16.7°F.). He also gives some data on the freezing points of different sugar solutions. Pickering (2) has also given some freezing-point data. Certain difficulties are encountered in determining the freezing points of the highly viscous cane-sugar solutions; and since the measurements recorded in these papers were made before the development of accurate methods, Mr. P. N. Peter of these laboratories has redetermined the position of this curve. His data are plotted. There is a very fair agreement among the data of the three men.

A similar survey of the work published on cane-sugar solubility failed to disclose any measurements carried out at the lower temperatures. Such measurements also offer considerable difficulty owing to the high viscosity of these concentrated solutions, with the resultant tendency to supercooling, and especially with the remarkable tendency of cane sugar to resist crystallization.

Work is in progress to determine definitely the solubility of cane sugar in water at the lower temperatures. For the purpose of this paper, however, it will be sufficient to determine empirically the solubility of cane sugar, since there are other means of locating the eutectic of the cane-sugar solution.

Where S is the solubility of sugar in 100 parts of water and t is the temperature in degrees Centigrade, the empirical formula (3) is:

$$S = \frac{28162}{157.97 - t}$$

The solubility curve of cane sugar calculated from this formula is given in figure 1, together with the freezing-point curves. It will be seen that the solubility and freezing-point curves intersect at a temperature of about −12°C. (10.4°F.). This temperature, lower than that given by Guthrie, is sufficiently low for the holding of water ices, at least those of moderate sugar content.

It now remains to determine the accuracy of the figure. A rather simple method, and one often used in metallurgical work, is to determine the rate of cooling of a sugar solution with the idea that if sugar separates from it because of heat liberation incident to this reaction, the rate of cooling will also change. The reverse process can also be carried out, namely, to warm the frozen mass and determine the rate of warming. This procedure is perhaps the better one, since it is possible to supercool such a solution, and the sugar separation may not come at the true eutectic. This is not the case with the warming process since, if equilibrium is always attained in the separation of the ice (and there is every reason to believe that it is), the sugar can not dissolve until the eutectic is reached. In this work, as clear-cut results are not to be expected as are usually obtained in metallurgical studies because of the comparatively poor heat conductivity of the sugar solutions and the slow crystallization or resolution of the cane sugar. The method is of value, however, as the results show.

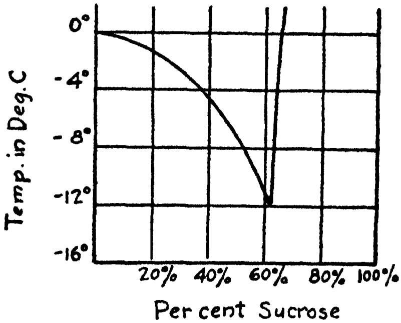

FIG. 1. SOLUBILITY AND FREEZING POINT CURVES OF SUCROSE-WATER SOLUTIONS

The experimental procedure was as follows: About 20 pounds of cane-sugar solution containing 25 per cent sugar was placed in the hardening room after freezing in the regular power ice-cream freezer. About 0.5 per cent gelatin had been added, which was sufficient to permit whipping in the freezer. In a liquid mass the ice crystals could concentrate on the surface of the mix, while here the gelatin gave the mass enough structure so that no such separation could take place. The temperatures in the center of the can were measured with a thermocouple with an accuracy of about one-fiftieth of a degree, the temperature being measured at varying intervals from five minutes to one-half hour, depending upon the rapidity with which the material was found to be cooling. The curve is given in figure 2*a*. It will be noticed that there is a distinct change of slope at a temperature of about −12°C. (10.4°F.). The warming curve also shows a change of slope at this temperature, and it is justifiable to conclude that this is the true eutectic temperature for cane-sugar solutions in water. In this particular instance, while some of the sugar had crystallized, the sugar separation was only partial, since the author was able to remove a small portion of unfrozen material from the mass when it was at a temperature of about 0°F., which substance showed a concentration of 73 per cent sugar. This represents a point in the freezing-point curve in the supersaturated

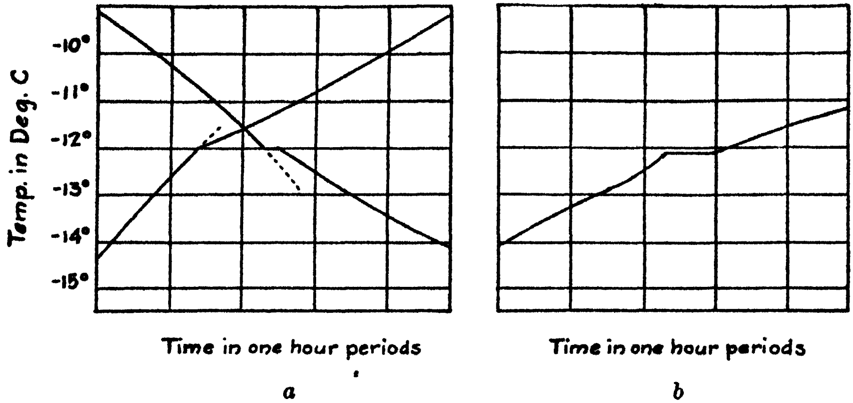

FIG. 2. *a*, COOLING AND WARMING CURVES OF WATER ICE; *b*, WARMING CURVES OF ICE CREAM

area, which is very good proof that ice crystallization reached equilibrium at all times during the experiment.

Figure 2*b* also shows the warming curve for an ice-cream mix. This curve is given because it shows that with a considerable sugar separation the temperature of the mass remained unchanged for nearly three-quarters of an hour. The discussion of the separation of milk sugar and cane sugar from ice cream will, however, be the subject of another paper. It should be noted here that the crystallization, due to the presence of substances other than water and sugar, takes place at a slightly lower temperature in the ice-cream mix than in the water ice.

In conclusion it may be stated that if water ice is not cooled to a temperature equal or below −12°C. (10.4°F.), sucrose can not separate from the solution. It may be well to emphasize that the value of this temperature is in no way dependent upon the original concentration of the sugar solution, also that it will in all probability be altered but little by the flavoring material added to the water ice. The probability of crystallization after this temperature is attained will undoubtedly be greater with the more highly concentrated sugar solutions.

The author is indebted to Mr. O. E. Williams for aid and advice with the work.

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BUFFERS OF MILK AND BUFFER VALUE*

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Since buffers are considered as substances which, by their presence in solution, increase the amount of alkali or acid that must be added to cause unit change in pH, the question of their occurrence in milk would be important. Clark (1) gives the following with regard to buffers: "By buffer action, we mean the ability of a solution to resist change in pH through the addition or loss of alkali or acid."

Among the agents, which act well as buffers, are mixtures of weak acids or bases and their salts. Milk might be expected to have a definite buffer value, due probably to the combined effects of salts and protein matter present. Van Slyke (2) has shown that human blood has a rather definite buffer value of 0.0228 at pH of 7.4. Milk is known to bear a close relationship to blood. Both have a small amount of sugars and protein in true or colloidal solution. These together with the inorganic salts are definite enough in quantity so that both milk and blood exhibit a definite osmotic pressure as measured by the freezing point. Since blood is well buffered against rapid change in pH it might be considered that milk would also show a definite buffering effect.

The importance of the buffer value of milk may be illustrated in a number of ways. In bacteriological experiments using milk as a media, if milk should have a constant or uniform buffer value, the rate of growth of bacteria could be observed by noting the acidity produced. However, if milk does not possess a very uniform buffer value at any pH range (5), the extent of growth could not be accurately made without, in each case, determining the buffer value of milk used. In a similar way the quantitative

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estimation of the various sugars in milk (3, 4), in which acidity produced by microörganisms is taken as the measure of the amount of carbohydrate present, would be very difficult, unless it were known that milk had a definite buffer value. Also, if different milk samples possessed widely varying buffer values, it would be possible for a highly buffered milk to remain sweet longer than a more poorly buffered one. It would necessarily take a larger amount of lactic acid to produce the same change in pH in the former as compared to the latter milk. Again, indefinite as the chemistry of milk may be, a number of investigators (6, 7, 8, 9, 10) seem to agree that acid phosphates and casein are responsible for the greatest part of the original acidity of milk. Granting these to be the chief causes of the original acidity of milk, it is reasonable to assume that a high acid milk should exhibit a greater buffer action than a low acid milk (11) because of the greater phosphate and casein content.

In this work Van Slyke's (2) method of measuring buffer values was used. A convenient quantity of the solution to be tested was taken and the pH measured. Strong acid or alkali, usually 0.5 N KOH or HCl, was added in small measured amounts and the pH measured after each addition. The buffer value was obtained from these data by plotting the amount of acid or alkali used against the change in pH produced. If too large a range is not taken, this average buffer value represents closely the actual buffer value at any point in that small range. As a numerical measure of the buffer value β of a solution, Van Slyke (2) proposes to take the number of gram equivalents of strong alkali or acid taken up by a liter of solution per unit change in pH. Since β varies with varying pH, the value at any given pH is defined by the ratio $dB/d\text{pH} = \beta$ expressing the relationship between the increment (in gram equivalents per liter) of strong base or acid B added to a buffer solution and the resultant increment in pH.

An example of the calculation of β is given to illustrate the method of evaluating the constant.

From figure 1 the following values may be taken from the

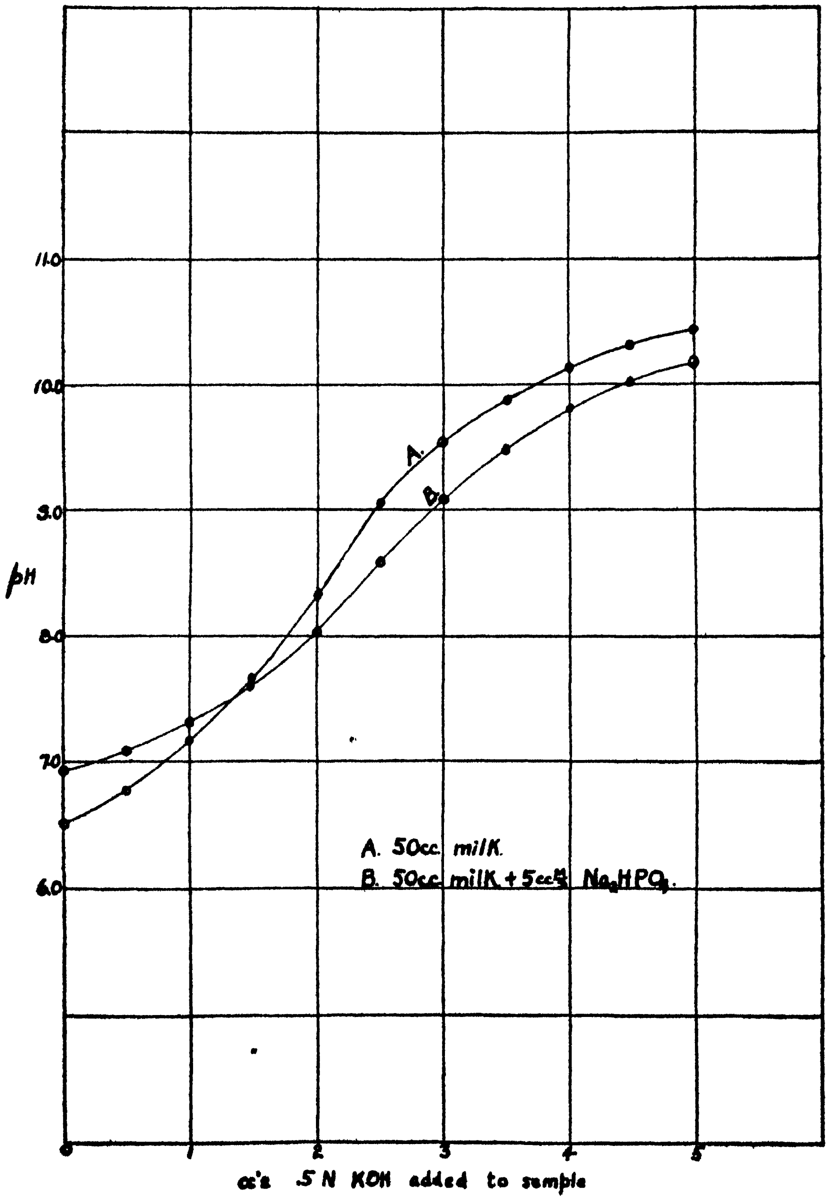
11.0
10.0
9.0
pH
8.0
7.0
6.0
A.
B.
A. 50cc. milK.
B. 50cc milK + 5cc M/3 Na2HPO4.
0
1
2
3
4
5
cc's .5 N KOH added to sample

FIG. 1

curve A. For the pH range 7.0 to 7.5 it required 0.58 cc. of 0.5 N KOH to cause a change of 0.5 pH in a 50 cc. sample of milk.

$$\frac{dB}{d\text{pH}} = \frac{\left(\frac{0.58}{1000} \times 0.5\right)}{0.5} \left(\frac{1000}{50}\right) = 0.0116 = \beta$$

where dB is the fraction of a gram equivalent of KOH added, $d\text{pH} = 0.5$ and 1000/50 is the ratio to change the experimental results from a 50 cc. sample of buffer solution to a 1 liter quantity. This value β is always a positive one, for if we add strong acid, it is equivalent to adding a negative amount of base. The pH increase is also negative, hence β is positive. In these terms a solution has a buffer value of 1 if a liter of that solution requires 1 gram equivalent of strong acid or base per unit change of pH.

The apparatus employed was the regular Leeds and Northrup potentiometer using the customary set-up for H-ion determination. The Hildebrand hydrogen electrode and a normal calomel electrode were used in all of the determinations. Additions of acid or base were made from a calibrated burette having a long, fine, capillary tip (12) reaching beneath the surface of the liquid at a point near the stirrer. This, together with the uniform rate of stirring provided by the motor-driven stirrer, prevented the possibilities of any "local action," and insured a rapid and even distribution throughout the liquid. Best results were obtained with a moderate rate of stirring with a stirrer shaped so that there was little churning action.

RELATIONSHIP BETWEEN pH REGION AND BUFFER VALUE

Table 1 gives a summary of the buffer values calculated from the data obtained over a pH range varying from pH 4.5 to pH 10.0. It may be observed from the table that the buffer value is not very definite in the acid range. In the alkaline range the value is more constant giving in the range pH 8.5 to 9.0 a value of 0.0067. The values given in table 1 as well as all values considered were on unpasteurized milk.

EFFECT OF ADDED WATER ON BUFFER VALUE

If we assume a definite buffer value in the pH range 8.5 to 9.0, the determination of this value would serve as a method of detecting added water in milk providing a variation from this value would be produced by dilution with water. Table 2 gives data from which the conclusion would be drawn that dilution of milk up to 30 per cent with water does not affect the buffer value. No error would then be introduced due to the dilution of the milk with the reagent required to bring the pH to a definite range.

TABLE 1

Variation of buffer value with pH region

pH ZONE	AVERAGE	LIMITING VALUES	VARIATION FROM AVERAGE	
4.5 to 5.0	0.03250	0.0379 to 0.0264	+0.0054	−0.0061
5.0 to 5.5	0.03255	0.0392 to 0.0280	0.0067	0.0045
5.5 to 6.0	0.02033	0.0234 to 0.0139	0.0031	0.0064
6.0 to 6.5	0.01720	0.0200 to 0.0148	0.0028	0.0024
6.5 to 7.0	0.01574	0.0165 to 0.0143	0.0008	0.0014
7.0 to 7.5	0.01200	0.0130 to 0.0112	0.0010	0.0008
7.5 to 8.0	0.00796	0.0094 to 0.0069	0.0013	0.0010
8.0 to 8.5	0.00635	0.0082 to 0.0060	0.0018	0.0004
8.5 to 9.0	0.00670	0.0074 to 0.0064	0.0007	0.0003
9.0 to 9.5	0.00950	0.0118 to 0.0082	0.0023	0.0013
9.5 to 10.0	0.01520	0.0174 to 0.0134	0.0022	0.0018

TABLE 2

Effect of added water on buffer value

	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5
Volume of water added to 50 cc. of milk	10 cc	10 cc.	15 cc	15 cc.	15 cc
For pH range 6.5 to 7.0	0.0160	0.0060	0.0165	0.0165	0.0174
7.0 to 7.5	0.0114	0.0115	0.0123	0.0116	0.0119
7.5 to 8.0	0.0080	0.0079	0.0075	0.0080	0.0080
8.0 to 8.5	0.0060	0.0062	0.0072	0.0066	0.0064
8.5 to 8.9	0.0068	0.0068	0.0074	0.0070	0.0068
9.0 to 9.5	0.0096	0.0098	0.0100	0.0098	0.0102
9.5 to 10.0	0.0154	0.0158	0.0176	0.0170	0.0170

BUFFER EFFECT DUE TO CASEIN

In the determining of the buffer values in the acid range, it was found that the casein was precipitated in the range pH 4.5 to 5.0, usually at about pH 4.7. No marked change in buffer value was observed in this range. Hence it would be concluded that the casein exerted very little influence as a buffer in this pH range in milk. Bailey and Peterson (13) noticed but little change in buffer values of flour extracts on the precipitation of the protein matter.

TABLE 3

Effect of added Na_2HPO_4 on buffer value

	SAMPLE 6	SAMPLE 7	SAMPLE 8	SAMPLE 9	SAMPLE 10	SAMPLE 11	SAMPLE 12	SAMPLE 13
Quantity of Na_2HPO_4 added to 50 cc. milk	5 cc M/3 solution	5 cc. M/3 solution	5 cc. M/3 solution	2 5 cc. M/3 solution	2 5 cc. M/3 solution	2 5 cc. M/3 solution	1 cc M/3 solution	1 cc. M/3 solution
pH range 6 5 to 7 0				0 0262	0 0240	0.0237	0 0192	0 0189
7 0 to 7 5	0 0222	0 0190	0 0212	0 0168	0 0170	0 0174	0 0138	0 0144
7 5 to 8 0	0 0122	0 0142	0 0132	0 0096	0 0108	0 0108	0 0092	0 0086
8 0 to 8 5	0 0092	0 0102	0 0090	0 0082	0 0084	0 0084	0 0074	0 0086
8 5 to 9 0	0 0094	0 0094	0 0094	0 0086	0 0088	0 0086	0 0078	0 0074
9 0 to 9 5	0 0130	0 0126	0 0122	0 0118	0 0118	0 0114	0 0114	
9 5 to 10 0	0 0190	0 0150	0 0200	0 0200	0 0196	0 0200	0 0172	0 0178
pH after addition of the Na_2HPO_4	6 924	6 917	6 916	6 681	6 741	6 741	6 668	6 650

BUFFER EFFECT DUE TO PHOSPHATES

In figure 1 are given the titration curves of 50 cc. of milk titrated with 0.5 N KOH and of 50 cc. of milk + 5 cc. M/3 Na_2HPO_4. The similarity of these curves to the curve obtained on titration of a solution of disodium phosphate is easily recognized, and suggests the possibility of the phosphates exerting a buffer effect in milk. To give information on this point, small quantities of Na_2HPO_4 were added to milk and the buffer values calculated over a range of pH 6.5 to 10.0. These values are shown in table 3. The average initial acidity of all samples of milk tested was between pH 6.500 and 6.630. By the addition of the phosphate the initial acidity of the milk was decreased.

The buffer value is also slightly increased depending upon the quantity of the phosphate added. For example, the average buffer value for the range 7.0 to 7.5 is 0.0120. The addition of 5 cc. M/3 Na_2HPO_4 increases β to 0.0212 while 2.5 cc. increases the value to 0.0170 and 1 cc. to 0.0140. Since the type of curve, as shown in figure 1, is the same and is only modified by having its values farther up on the scale, it indicates for this range a very close relation between phosphate and the buffer substances present in milk.

As Na_2HPO_4 in water solution hydrolyzes to give a basic reaction, that largely accounts for the increase in initial pH and very probably for the increased buffer action. It was thought possible that the results observed with Na_2HPO_4 might simply be a salt effect on the buffers contained in milk, that is, that any salt might modify the buffer values in the different ranges in somewhat the same manner. On the addition of KCl, $CaCl_2$, and $FeCl_3$, to samples of milk it was found that while the buffer value was affected, the results were neither regular nor in the same order as with the Na_2HPO_4. Hence the effect of Na_2HPO_4 would not be considered as a salt effect upon the buffers of milk.

CONCLUSIONS

1. The calculated buffer value of milk varies with the pH range and is the more definite at the range pH 8.5 to 9.0, with an average value of 0.0067.

2. Casein has very little effect as a buffer in the region of its iso-electric point, since the precipitation of the casein has no great influence upon the buffer value.

3. The phosphates of milk are at least among the most important buffers of milk. This is indicated by the fact that there is a great similarity between the titration curves of milk and Na_2HPO_4.

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A VOLUME WEIGHT STUDY OF ICE CREAM*

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INTRODUCTION

The problem concerning the shrinkage of ice cream upon its being dished from bulk can containers is an ever-present one whenever and wherever ice cream is sold in bulk to the consuming public. This problem is assuming greater importance daily as the ice cream industry is growing so rapidly. With its direct bearing upon the increasing agitation now in progress for a sales by weight program and for goods packaged at the manufacturer's plant, it has indeed reached a point of very vital significance to the industry.

It has long been observed that there is considerable shrinkage in volume when ice cream is dipped from a can. Numerous articles have been written and published upon this problem. The latest and most important publications are here given as references (1–8).

The purpose of this research was to ascertain the effect of various factors on shrinkage with a view of discovering possible preventive methods of diminishing shrinkage.

EXPERIMENTAL

Method

In the scientific study of any problem there must be a standard upon which to base results. This is particularly true of any work upon ice cream which is of such a complex nature. So

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standard methods of procedure were employed throughout the entire work.

A standard mix containing 12 per cent fat, 10.5 per cent serum solids, 38 per cent total solids, 14.5 per cent sugar, 0.5 per cent gelatine and 0.5 per cent powdered egg yolk was used in all work except as otherwise noted in the text. The mix was made from fresh cream, skim milk and skim milk powder.

The average weight in pounds per gallon of the standard mixes was 9.16.

All freezing was done in a Fort Atkinson freezer of 10 gallons capacity. Fifty pounds of mix were used for each batch. The ice cream was drawn into 3-gallon cans. These cans were tared so that a definite check on the overrun could be obtained, for each can was reweighed after filling and the overrun thus accurately determined. The overruns thus determined were the ones used in all calculations.

An interesting result of this weighing of each can is observed when the overrun of the first, second, and third cans drawn are compared. The results show that in 65 per cent of the cases the second can was of higher overrun than the first can and in 30 per cent of the cases the second can was of lower overrun. With the third can the ratio was 50 per cent higher and 45 per cent lower overrun than in the first can. The can of the highest overrun was the first can in 9 per cent of the cases, the second can in 24 per cent of the cases and the third can in 18 per cent of the cases. The figures for the lowest overrun are 23 per cent for the first can, 7 per cent for the second and 19 per cent for the third. These figures are from observations on 58 batches which gave 174 cans.

The overrun was tested frequently during the whipping by running out the ice cream into a one pint cup measuring $2\frac{1}{16}$ inches deep by $4\frac{1}{16}$ inches in diameter. The excess ice cream was leveled off by means of a saptula. The large diameter of the cup permitted the ice cream ribbon to fold into the container with a minimum amount of included air holes. The cup and contents were weighed on beam scales and the overrun computed directly. As previously mentioned, the overrun was checked by weighing each individual can.

Before dishing, each can was removed from the hardening room and placed in a mechanically refrigerated cabinet where it was allowed to temper for at least twelve hours. Unless otherwise stated all cans were tempered and dished at 10°F. When removed from the cabinet, the can was immediately placed into an insulated jacket and remained therein during the entire period of dishing.

The temperature of the ice cream was taken with a low reading thermometer from time to time and no appreciable change in temperature was noted except in a few cases when the work was interrupted.

In all the work every factor was kept as nearly uniform and standard as was possible, varying but one factor at a time in order that its effect alone might be studied. The matter of personal equation was kept as nearly constant as could be desired by using great care in all phases of the work. The pressure exerted upon the dishing tool, the degree of heaping the disher, the pressure applied to the ice cream as it was introduced into the carton, all were carried out with extreme uniformity. Yet at the same time conditions were strived for which were as nearly as possible identical with those which would be met with in actual commercial work. The data for each can were never reviewed until after the entire can had been dished.

A. Size and type of dishing tool

In order to study the effect of the size and type of disher employed for the dishing of the ice cream, several sizes and types were used in these experiments. The regular self ejecting semi-spherical disher was used in most work. Wherever the word "disher" appears, this type of disher is indicated. The size number indicates the fraction of a quart contained in one level disherful.

When dishing the ice cream, the number of disherfuls per 3-gallon can was noted. Each disherful was placed upon a clean tared paper plate and weighed.

In all dishing, attempt was made not to get too many extra full dishers or too many with air spaces in them. To make a solid, even, good looking ball such as commercial dealers would dish, the ice cream was pressed into the disher by a slight, yet consistent, pressure against the side of the can. The excess ice cream was always removed by a twisting motion against the side of the can. When an air hole in the disherful was attempted to be filled, an extra heavy disherful always resulted. It was also noticed that a ball showing an air space was not necessarily lighter than one which had a perfect semi-round shape.

The average data obtained when using the semi-round dishers is given in table 1. It will be noted from this table that the volume shrinkage is practically the same for all sizes of dishers.

TABLE 1

Effect of size of disher

SIZE OF DISHER	CAN			DISHERFULS		EQUIVALENTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Average weight	Quarts from can	Dishers from a quart	
		pounds	*per cent*		*ounces*			
No. 10	4	13 7	97	70	3.1	7.0	5.8	42
No. 12	12	14 0	99	81	2 6	6.9	6.9	44
No. 16	4	14 2	93	117	1.9	7.4	9.8	36
No. 20	3	14 0	93	131	1 7	6.8	10 9	44
No. 24	6	14 1	97	170	1.3	7.0	14.1	41
No. 30	3	13 9	98	209	1 0	6.9	17.1	42
No. 10	*1*	*13.6*	*105*	*94*	*2 0*	*9.4*	*7.8*	*22*
No. 20	*1*	*13.2*	*110*	*167*	*1.2*	*8.3*	*13.8*	*31*

This may be explained in that the larger scoops require more pressure to fill them, where-upon the air is squeezed out to a certain degree, and while the smaller scoops require less pressure per scoopful, nevertheless they require this smaller pressure to be applied to a smaller volume of ice cream, and also this smaller pressure must be applied more often or more times per each can dished. Therefore the effect of the smaller pressure is more additive and equals the larger pressure when the whole can is considered.

In order to obtain data which might show the effect of heaping the scoop, two cans were dished and each disherful leveled off with a spatula. The results are added at the foot of table 1 and are indicated by italics. These figures are of interest because the variations in the sizes of the disherfuls should have been nil. They also show that a considerable part of the shrinkage was due to the heaping of the scoops. This factor will be considered again later when the results of loading the ice cream into cartons are given.

Two balls dished from the same can and using the same size scoop varied at times as much as 2 ounces in weight.

Averaging the weights of the disherfuls from the top third, the middle third and the lower third of the can, no difference was noted. This indicated that the ice cream remaining in the can was not compressed by the dishing out of that above it. This held throughout the entire work. That this condition would not have held with all operators is fairly certain.

When other tools than the dishers were used for dishing, the ice cream had to be loaded into cartons in order to have any figures relative to shrinkage. The pint tapered carton was arbitrarily selected for a standard.

The ice cream spoon had a 2¾ by 3½ inches bowl with a somewhat square end and a short wooden handle. The "trowel" was of aluminium, such as is used for leveling ice cream in slab moulds. The "trowel" was 4½ inches long by 3 inches wide and had its outer end curved somewhat to fit the curvature of the can. The "round loader" was one now on the market which cuts a cylindrical cake of ice cream from the can. The "square loader" was also one now on the market which cuts a square cake from the can. Both of these loaders are well known to the trade.

In certain instances the shrinkage when computed from the number of scoops obtained from a can was more than was indicated when this same ice cream was placed in the cartons. It may at first seem strange that the shrinkage could decrease upon further handling. But, as may readily be concluded from a careful consideration of the conditions, this decrease in shrinkage should have been expected because the ice cream balls when placed in the carton do not fit evenly and exactly together.

The air spaces remaining account for the increase in volume. And some of these air spaces are bound to remain, regardless of the pressure used in pushing the ice cream into the container. Ice cream once compressed would resist more strenuously any further compression. Many of the cartons were stripped in order to observe the number and sizes of these air spaces. This was done with cartons filled by all the tools. Aim was made to fill all cartons to a degree of firmness that would satisfy the retail trade. Consistent filling was at all times a chief objective. Care was likewise expended that the work did not become fussy to the extent that it would not approximate trade conditions.

TABLE 2

Effect of type of tool

TOOLS	CAN			PINT TAPERED CARTONS		VOLUME PER CENT SHRINKAGE
	Number	Net weight	Overrun	Number from can	Average weight	
		pounds	*per cent*		*ounces*	
Disher all sizes, average	9	14.0	95	14.1	15.2	39
Spoon	7	13.9	96	16.6	12.9	30
Trowel	5	14.0	94	17.3	12.7	28
Round loader	3	14.5	91	20.0	10.5	16.7
Square loader					9.9	

The weight of the pints filled by the dishers was influenced a great deal by the manner in which the first few balls were placed into the carton. The bottom of the carton being narrower, the balls did not fit together as snugly. Successive balls were placed in alternate sides of the carton. Table 2 gives the results when different types of tools were used. Employing the ice cream spoon as the dishing tool, less shrinkage resulted than when the dishers were used. In dishing with the ladle, care was exercised not to take too large ladlefuls. If they were too large, more pressure was required to push the ice cream into the carton and the less likely were the air spaces to be filled, even though the greater pressure was applied. Also if too small a spoonful was taken each time, the ice cream received more intensive handling and therefore lost more of its overrun.

Most of the cartons were carefully examined for air spaces. This was accomplished by submerging the carton in hot water for a moment and then inverting it over a paper plate, whereupon the ice cream fell out intact as a cake and was easily observed. All the cakes so examined were found to be free from air holes which were larger than would be tolerated by the most exacting customer.

In the use of the trowel the same general procedure was followed and the same factors were involved as applied to dishing by use of the spoon. With the trowel, however, it was easier to cut a cake of ice cream which more nearly fitted into the carton. This enabled the operator to dish ice cream into the cartons and have the ice cream retain more nearly the properties it had when in the can. A special case in which the carton is of the round type, will be given later when the effect of the type of carton is considered. The trowel and round carton were the best combination.

For filling the tapered cartons, the trowel should be wide as the top of the carton. This allowed a better filling of the carton for as the ice cream seated itself, it conformed snugly to the shape of the carton and filled the corners nicely without having to be pushed and crowded by excessive pressure.

Very little success was obtained from use of any of the semi-mechanical loaders, so designed as to cut a cake of ice cream to fit exactly into the particular carton for which the loader was adapted.

The loaders cut the ice cream from the can without altering the condition of the ice cream in any way. Thus the lightest weight pints were obtained by use of these dishers which dished the ice cream with very little shrinkage. However the author believes the objections to their use offset the advantages. All such dishers needed to be dipped into water after each time used. This was messy at best. The wet tool dripped water into the can which water would later freeze and give a coarse or lumpy texture to the ice cream subsequently dished. These tools having a large surface area, considerable water was therefore carried by them. Also considerable ice cream was carried to the wash water. So, after two or three usages, the water became quite dirty. When this water dripped upon floor and cabinet the messy condition constantly increased. The wash water being relatively warm at best, would offer a good place for the culture of bacteria which would eventually be carried to the ice cream.

These loaders were necessarily of small enough dimensions to allow of their introduction into the container before the expulsion of the ice cream. Consequently the cake of ice cream was not as wide as the carton and an air space was left between the ice cream and the carton. Although the disher did dish a true pint, nevertheless the carton of ice cream did not look full and actually the ice cream did not measure a full pint if much pressure was used in expelling it. If the tool was allowed to get too cold, the pressure necessary for ejection became very great.

With the round type of tool, the ice cream cake could be broken off at the bottom from that remaining in the can by a twisting motion. However with the square instrument this could not be accomplished as easily. Only with intense rocking could the cake be freed from the remaining ice cream. This procedure necessarily squeezed and compressed the ice cream adjacent to the loader in position.

The temperature of the loaders needed careful regulation which was impractical. If too cold, ice cream stuck to the outside and great pressure was needed to expel the cake. If too warm, the cake often would not break at the bottom.

The data for all cartons filled showed that there was no perceptible increase in weight of the cartons filled from the bottom of the can over those filled from the top. Thus it would seem that there was no compression of the ice cream when the upper portions were dished.

B. *Effect of overrun*

The relation of overrun or per cent of swell would seem of utmost importance in a study of shrinkage from dishing manipulations because the shrinkage in volume is due to the squeezing out of some of the air incorporated into the ice cream during the freezing process. One would naturally infer that the larger amount of air so incorporated would result in a greater shrinkage when that particular ice cream was dished. That the results of this investigation do not uphold such a conjecture may seem rather startling at first but when the factors involved are carefully studied, the data here cited would be expected.

From the data in tables 3 and 4 it is readily seen that the overrun has hardly any effect upon the number of pints obtained, namely the shrinkage, but it does, however, govern their weight.

TABLE 3

Influence of overrun on number of disherfuls

TOOL	CAN			DISHERFULS		EQUIVALENTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Average weight	Quarts from can	Dishers from quart	
		pounds	*per cent*		*ounces*			
Disher No. 10	4	13.7	97	70	3.1	7.0	5.8	42
	2	13.1	112	78	2.8	7.3	6.5	34
Disher No. 12	12	14.0	99	81	2.6	6.9	6.9	44
	4	15.2	80	84	2.8	7.0	7.0	41
Disher No. 16	4	14.2	93	117	1.9	7.4	9.8	36
	1	15.2	77	131	1.89	8.2	10.9	32
Disher No. 20	3	14.0	93	131	1.7	6.8	10.9	44
	1	13.3	107	133	1.58	6.7	11.1	44

This fact is in accord with the findings of Ruehe (1) but is not substantiated by the work of Fisher and Judkins (2), or by Bierman (5). It is of interest that one of these investigators dished at this laboratory 18$\frac{1}{3}$ pints from a can of 85 per cent overrun and 16$\frac{4}{5}$ pints from a can of 125 per cent overrun, both cans containing ice cream from the same mix.

The fact that the overrun was found to play such a minor rôle as regards shrinkage may be explained in that the greater the percentage of overrun, the more pliable the ice cream became, and less pressure was required to fill the dishing utensil and the carton. There was slightly more shrinkage in cans of very high overrun as compared with cans of very low overrun. There was probably a difference of about 1 pint at most in amount of ice cream dished for overruns between 60 per cent and 120 per cent, a range of 60 per cent in overrun. For any ordinary ranges in overrun the shrinkage differences were negligible.

TABLE 4

Influence of overrun on number of pint cartons filled

TOOL	CAN			PINTS OBTAINED		VOLUME PER CENT SHRINKAGE	TYPE CARTON USED
	Number	Net weight	Over-run	Number	Average weight		
		pounds	*per cent*		*ounces*		
All Dishers	9	14.0	95	14 1	15.2	39	Pint tapered
No. 12	E241	13 7	102	14.0	15.6	42	Quart tapered
	F333	14.9	81	14 0	17.0	42	Quart tapered
	F343	13 3	103	13 0	15.6	46	½ pint tapered
	F321	14.8	82	14 0	16 8	42	½ pint tapered
Spoon	7	13.9	96	16.6	12.9	30	Pint tapered
	C142	12 7	112	18 0	10.8	25	Pint tapered
	C121	13 2	105	19 0	11.2	24	Pint tapered
	B101	15 9	75	18.0	14.2	25	Pint tapered
	E312	15 6	77	18.0	14.1	25	Pint tapered
Trowel	5	14 0	94	17 3	12 7	28	Pint tapered
	F372	12.1	120	17 0	11.0	29	Pint tapered
	E303	13.4	106	16 0	13.2	33	Pint tapered
	F352	14 9	81	17 0	14.2	29	Pint tapered
	F323	16 1	67	17 0	14 7	29	Pint tapered
	F373	12.3	120	20 0	9.6	17	Pint round
	E282	14.6	90	20.0	11.5	17	Pint round

Again emphasis is put upon the fact that all the work was done in as uniform and constant manner as was possible in order that the effect of the overrun and of that one factor alone might be studied. In actual dishing by the average retailer he would find the high overrun would compress easier and therefore give it more severe treatment than he would to ice cream of harder consistency.

C. A study of personal equation

When the results of work done by the author on the influence of overrun were reviewed, it was decided to have cans of varying overrun dished by different operators. The results of this phase of the investigation are listed in table 5. It is of interest to note that the average results are consistent with those obtained by the author. The same operator, even when experienced, will very likely get a difference of one pint or even more when dishing in like manner two cans containing identically the same ice cream. This will account for the occasional slight variation from dishing cans of ice cream from the same mix and batch and which have had exactly the same treatment.

TABLE 5

Results of different operators

OPERATOR	OVERRUN	TAPERED PINTS	AVERAGE WEIGHT	REMARKS
			ounces	
A	95	18.0	11.9	Good looking packages
	125	18.5	10.1	
B	95	19.0	11.5	Too careful; long time; small spoonfuls
	125	17.6	11.0	
C	95	15.0	14.1	Pressed too hard
	125	15 5	12.4	
D	85	18.3	12.6	Difference of 40 per cent in swell
	125	16.8	10.8	
E	90	18.0	13.1	Very poor; air spaces
	125	13.5	14.5	
F	67	17.0	14.7	Typical for author; difference of 53 per cent in swell
	120	17.0	11.0	

Average difference low and high overrun = 1.1 pints more from low.

D. Size and shape of carton

In order to study all factors encountered in actual trade conditions, experiments were conducted in which the ice cream was dished into cartons of varying sizes and of various styles.

As is shown by table 6, the size of the carton does not influence the shrinkage except to very slight degree. In general the smaller the size of the carton, the greater was the shrinkage observed in dishing yet this difference was not very great, amounting on the average to about one-half pint per can between quart and one-half pint size cartons.

TABLE 6

Influence of size of carton

TOOL	CAN			CARTONS FILLED			EQUIVALENT		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Size	Number	Weight	Number of pints	Weight of pint	
						ounces			
Disher No. 12	9	14.0	95	Pints	14.1	15.2	14.1	15.2	39
	3	13.8	95	Quart	7	32.0	14.0	16.0	42
	3	13.9	96	½ pint	27	7.9	15.5	13.8	44
Spoon	7	13 9	96	Pints	16 6	12.9	16.6	12.9	30
	2	13.8	95	Quart	8.5	25.9	17.0	12.9	29
	3	14.6	88	½ pint	33	6.6	17.3	13 2	28
Trowel	5	14.0	94	Pints	17.3	12.7	17.3	12.7	28
	2	14.2	95	Quart	8.5	26.3	17.0	13.2	29

The lack of any marked variation in shrinkage due to the size of carton used together with the uniform shrinkage noted when the disherfuls were and were not placed in cartons seems to indicate that the most of the shrinkage was due to getting the ice cream out of the can and not due very greatly to the process of packing it into the carton. The differences which were observed were probably due to the tendency of the larger cartons to bulge at the sides. Also it may have been due somewhat to the fact that the disherfuls fitted in different manner into the different sizes of cartons.

The results of work using the round and the tapered carton which are listed in table 7 showed that there was practically no difference in the effect of the type of carton upon shrinkage except in the special case when the trowel and the round carton were employed. This was due to manipulation. The trowel was used to cut a large cake of ice cream from the can. Then using the round carton as a sort of cookie cutter, it was pressed down over the ice cream held on the trowel. In this way a cake of ice cream was cut out which just fitted into the round carton. The sides of the round carton being stiff, this procedure was an easy one. Two such cuts usually filled the round carton very nicely and by twisting at the end of the second cut, a nice smooth surface, flush with the edge of the carton was obtained. Thus it was found possible to dish the ice cream with very little shrinkage when this combination of tools was used. This furnishes an ideal method for dishing ice cream.

TABLE 7

Influence of type of carton

TOOL	CAN			PINT CARTON			VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Style	Number	Weight	
		pounds				*ounces*	
Disher No. 12	9	14.0	95	Tapered	14.1	15.2	39
	3	13.7	97	Round	14.6	14.8	39
Spoon	7	13.9	96	Tapered	16.6	12.9	30
	4	13.5	98	Round	16.9	12.8	30
Trowel	5	14.0	94	Tapered	17.3	12.7	28
	3	13.7	100	Round	20.0	10.7	17

Note effect of trowel with round carton!

E. Effect of dishing temperature

Conditions surrounding the retailing of ice cream vary greatly but probably the greatest variable factor is the temperature of the cabinet. This has been in the past particularly true although the widespread introduction of the mechanically refrigerated cabinets and other improved types, have done much to remedy the poor temperature conditions, still there is great interest in the proper temperature for dishing and the relation between temperature and shrinkage.

During the early work of obtaining the number of disherfuls from cans, when each disherful was weighed, it was noticed that the individual disherfuls became heavier if the ice cream became very soft or if it became very hard. The soft ice cream evidently filled the disher more solidly while the hard ice cream required more pressure to get the disherful and therefore suffered loss of air. These results however did not effect the weights or numbers of pint cartons filled because in pushing the ice cream into the cartons, the very soft ice cream could not be subjected to any great pressure, it being squeezed out it attempted, while the very hard ice cream would not pack under even severe pressure and therefore air spaces were left between the lumps. This reasoning applies to all ice cream dished at differing temperatures. Therefore the temperature equation becomes practically zero except for very wide variations. There were two factors working against each other, the firmness of the ice cream and the

TABLE 8

Effect of dishing temperature

TEMPERATURE	CAN			PINTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Weight	
°F.		*pounds*			*ounces*	
5	3	14 0	92	17.1	13 0	29
10	8	13.9	96	16 5	13.0	30
15	3	13 8	96	16.6	13.1	31
23	1	13.7	96	20 0	9 88	17

pressure necessary for proper dishing. These effects very nearly counterbalanced each other.

The data observed in this section of the research are given in table 8. The cans dished in this study were all tempered at the respective temperatures for at least twelve hours. All the dishing was done using the ice cream spoon and the pint tapered cartons. So it may be seen from this table that the temperature effect is practically zero except in very extreme cases.

The best dishing temperature was found to be 10°F.

F. Effect of varying composition

The next factor to be investigated was whether the composition of the mix might have some vital connection with the dealer's shrinkage. There was no marked differences in shrinkage from ice creams made from mixers of varying viscosities and acidities.

TABLE 9

Mix I—14.0 per cent fat, 38 per cent total solids

TOOL	CAN			PINTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Weight	
Disher No. 12	Average for standard mix from table 2					39
		pounds			*ounces*	
	3	13.7	96	14.1	15.2	41
Spoon	Average for standard mix from table 2					30
	3	13.8	97	17.3	12.7	28

For study of the influence of actual variation of composition, four special mixes of two hundred pounds each were prepared in which the percentages of fat, of total solids, and the amount of filter were varied one at a time and in which all other ingredients were kept standard. Mix IV contained no egg powder. All other mixes used in this research contained 0.5 per cent yellow egg powder.

As shown by data given in tables 9 and 10, the percentage of

TABLE 10

Mix II—8.0 per cent fat, 38 per cent total solids

TOOL	CAN			PINTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Weight	
Disher No. 12	Average for standard mix from table 2					39
		pounds			*ounces*	
	3	14.3	93	13.6	15.8	43
Spoon	Average for standard mix from table 2					30
	3	14.4	93	16.1	14.2	32

TABLE 11

Mix III—34.0 per cent solids, 12 per cent fat

TOOL	CAN			PINTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Weight	
Disher No. 12	Average for standard mix from table 2					39
		pounds			*ounces*	
	3	14.0	97	14.1	15.6	41
Spoon	Average for standard mix from table 2					30
	3	13.7	96	17.3	13.0	28

TABLE 12

Mix IV—no egg powder, 12 per cent fat, 38 per cent total solids

TOOL	CAN			PINTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Weight	
Disher No. 12	Average for standard mix from table 2					39
		pounds			*ounces*	
	3	14.4	93	14.1	16.2	41
Ladle	Average for standard mix from table 2					30
	3	14.2	95	17.0	13.2	29

fat plays a very minor rôle in the shrinkage even when the per cent of fat is varied between 8 and 14 per cent a difference of 6 per cent. The limits of 8 and 14 per cent were chosen because most ice cream today on the market would come within these percentages.

Next the per cent of total solids was varied by decreasing this ingredient 4 per cent. The results for mix III containing 34 per cent solids are given in table 11.

Here again a variation in composition, this time in the per cent of total solids cannot be attributed to causing any very marked change in amount of shrinkage.

The action and value of filler has always been of interest and so the next mix studied was made up with no egg powder. The results for mix IV containing no egg powder are given in table 12.

Yet again there is no noticeable difference whether the composition was altered, this time the egg seeming to have no great influence on shrinkage.

It is the opinion of the author, however, that all variations in composition do influence the properties of the ice cream, and so also the shrinkage upon dishing. The effect would have to be very marked indeed to show when a three gallon can was dished. But it is evident that these influences are of a very minor character when considered with the other factors such as the operator.

SUMMARY

1. The shrinkage of ice cream when dished from bulk containers averaged about one-third of its original volume.
2. The shrinkage was nearly the same regardless of the size or type of disher or the size and shape of the carton used.
3. The ice cream spoon was found to be the most practical dishing tool.
4. The effect of the overrun on the volume shrinkage was not very marked.
5. Method of dishing and filling of the cartons was ascribed as being of vital significance on the shrinkage encountered in commercial practices.

6. The effect of the dishing temperature was not very great unless extreme temperatures were encountered.

7. The composition of the mix did not influence the volume shrinkage sufficiently to be of importance when compared with item 5.

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A STATISTICAL STUDY OF CREAMERY OPERATION*

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Conclusions drawn from general observations by the persons engaged in an industry are not always true. Fortunately, the statisticians have developed methods of analyzing large numbers of figures so that it is possible to weigh fairly accurately the facts that a certain set of data contains.

The data on which this article is based were obtained by survey[1] from 78 creameries in Minnesota for the year 1919. All of these creameries were of the farmer type, such as is found in villages and in the smaller cities. None were centralizers. There were 71 farmers' coöperative plants, and 7 independent or proprietary plants.

There was a wide variation among these 78 creameries. Some were small and a few were large. Some were housed in old, worn-out frame buildings, but the majority were operating in new homes, most of which were constructed of brick. Some of the creameries were conveniently arranged, and a few were very inconvenient. Some had good accounting systems, and others had very poor records. Most of the creameries were efficiently administrated, but a few apparently were not managed at all,

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[1] The survey was made by the writer, who represented the Federal Bureau of Markets and who collaborated with Dr. John D. Black, Chief of the Division of Agricultural Economics of the University of Minnesota. In all, 102 creameries in 42 counties were visited. Some of the reports were not sufficiently complete to be used, and so only 88 creameries were included in the final analysis. The summary of that study was published in Technical Bulletin 26 of the University of Minnesota, 1924, entitled "Economic Aspects of Creamery Organization." The supply of that bulletin is now practically exhausted. The present article is a second study of some of the same data, but from a different angle. Certain data are missing in a few of the reports of the 88 creameries considered in the first study, and hence only 78 plants are included in the present analysis.

they ran themselves. Some had sufficient capital on which to do business, and others were "hard up." Some received good cream and took pride in making "higher scoring" butter, while others paid little attention to the quality of their product.

The relationships that existed between several of the important items in the manufacture of butter in these 78 creameries may be shown in different ways. The mean, the standard deviation, the coefficient of variability, the coefficient of correlation, and the probable error were calculated, and these all help to shed light on what actually took place.

The nine important factors in the manufacture of butter, which were studied, are:

x_1. Price paid for fat in cents. This is the price which the farmer received for his butterfat.

x_2. Price received for butter in cents. This is the price which the creamery received for the butter.

x_3. Score of butter. The writer scored the butter at the creamery. All of it was less than one week old.

x_4. Percentage overrun. Overrun is the increase in butter over the butterfat. Overrun may be defined as the sum of the casein, the salt, and the water in the butter, minus the losses in manufacturing. Good commercial creamery butter contains about 81 per cent of fat.

x_5. Cost of labor per hour, in cents. The value of the time of directors, secretary, treasurer, bookkeeper, manager, workmen in the plant, and workmen's compensation, is all included in this calculation.

x_6. Hours of labor to make 1000 pounds of butter. All of the labor within the creamery that went into the butter was recorded.

x_7. Pounds of butter made in 1919, in thousands. This is the volume, or amount, of butter manufactured in each creamery in 1919.

x_8. Building cost per 1000 pounds of butter, in cents. This item includes interest, maintenance, depreciation, taxes, and insurance, on the buildings. The annual net cost was divided by the annual production.

x_9. Equipment cost per 1000 pounds of butter, in cents. This factor includes interest, maintenance, depreciation, taxes, and insurance, on the equipment. The annual net cost was divided by the annual production.

MEAN, STANDARD DEVIATION, VARIABILITY, AND PROBABLE ERROR

The means and the variables for the nine factors in this study are given in table 1. The mean, or average, price paid to the farmers for butterfat, was 64.8 cents, whereas the price received for the butter was 56.4 cents. On the average, therefore, the 78 creameries paid 8.4 cents more for the butterfat than they received for the butter.

TABLE 1

Mean, standard deviation, and coefficient of variability

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
1. Price paid for fat, in cents . .	64 75± 0.30	3.91± 0 21	6.04±0.33
2. Price received for butter, in cents ..	56 36± 0 16	2 07± 0 11	3.68±0.20
3. Score of butter	91.23± 0.12	1.51± 0.08	1.65±0.09
4. Percentage overrun.	23.82± 0.14	1.83± 0.10	7.70±0.42
5. Cost of labor per hour, in cents . ..	44.38± 0.84	11.00± 0.59	24.78±1.42
6. Hours of labor to make 1000 pounds pounds of butter...	35.59± 1.15	15.13± 0.82	42.51±2.68
7. Pounds of butter made in 1919, in thousands...	230.21±11.64	152.39± 8.23	66.19±4 89
8. Building cost per 1000 pounds of butter, in cents.	603.56±25.85	338.53±18 28	56.08±3.86
9. Equipment cost per 1000 pounds of butter, in cents................. ..	420.67±13.83	181.08± 9.78	43.04±2.72

The average score was 91.2, which was fairly well toward "extras," the range being from 87 to 94.

The average overrun was 23.82 per cent, which is high. In passing, it should be stated that the high overrun obtained by many creameries was due to the practice of not weighing the half pounds. For illustration, if a can of cream weighed 35½ pounds the creamery would record it as 35 pounds. Thus the half pounds were not counted in the volume of butterfat, but they were included in the weight of the butter. It is apparent that the overrun would be increased thereby.

The average cost of labor in 1919 was 44.4 cents per hour, with a range from 26 to 80 cents. The hours of labor to make 1000 pounds of butter showed an average of 35.6, with a range from 16.1 to 85.2 hours. The volume of butter, or the amount manufactured was 230,218 pounds and ranged from 31,000 to 886,000 pounds. The average building cost was $6.04 per thousand pounds of butter manufactured, and the range was from $1.41 to $7.80. The equipment cost per thousand pounds of butter made was $4.21, with a range from 82 cents to $9.17.

One of the striking features of the study is the variability shown.[2] Statisticians have constructed an index of variability which, to speak figuratively, is nothing more than a thermometer showing how things vary. It is like "percentage" in that it quickly and clearly makes a comparison discernible; it also measures the difference in variation. The variation in score of butter is small, due to the limited range of butter scores. Butter scoring 86 is very poor, while a score of 94 indicates a very high grade of butter. The coefficient of variability for "score of butter" was 1.7. The most variable factor was "pounds of butter made in 1919, in thousands," the coefficient of which was 66.2.

The variability coefficient of the price paid for butterfat was 3.68, which was about twice that of the price received for the butter. The price which the creamery gets for butter is based on the market conditions and the grade of the butter, and, as has already been pointed out, the score of butter is very non-variable. The price which the farmer receives for the butterfat is more variable, because, in addition to the price of the butter, it is affected by many of the problems of manufacture, particularly the hours of labor consumed in making the butter, and the volume of output.

The cost of labor is about four times more variable than the price of fat. It is affected more by volume than by any other factor, although the hours of labor required to make a unit of butter is directly related.

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[2] Coefficient of variability $= \frac{\sigma}{Me} 100$.

CORRELATION

The explanation of the significance of the coefficient of correlation is very aptly expressed as follows by G. F. Warren[3] when he says:

> A coefficient of correlation furnishes a convenient means of expressing the relationship of one set of facts to another set. For example, the daughters of good cows tend to be, but are by no means sure to be, good cows. If it were an absolute certainty that every daughter of a cow would produce precisely the same amount of butterfat as her mother produced, the correlation would be expressed by 1. If there were not the slightest relationship between the amount of butterfat given by a cow and that given by her daughters, the correlation would be 0. Studies of butter production have shown that the correlation is 0.28. This shows that there is a considerable chance that the daughter of a cow that produced a large amount of butter will be a high producer, but that the results are far from certain. The relation of the heights of men to the heights of their sons shows a correlation of 0.51. . . . .
>
> Even the low correlation of 0.28 between the production of cows and that of their daughters is enough so that men are willing to pay more for the daughters of good cows than for daughters of poor cows.

The eight other variables were correlated with the price which the farmer received for the butterfat and with one another. The first line in table 2 shows the correlation coefficients between eight factors and the "price paid for butterfat." Likewise the second line correlates the seven factors with "price received for butter." In a similar way the coefficients of correlation given in the columns show the relationships between the column headings and the heading of the line on which the coefficients are located.

The correlation between the price received by the farmer for the butterfat, and the price obtained by the creamery for the butter, is very high ($r = 0.743$). The correlation between the price received by the farmer for the butterfat, and the score of the butter, also is high ($r = 0.631$). Overrun, on the other hand,

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[3] Prices of Farm Products in New York. Cornell University Agr. Exp. Sta. Bul. 416, 1923, p. 33.

TABLE 2

Correlations between nine factors in seventy-eight creameries in Minnesota, in 1919

	(1) PRICE PAID FOR FAT, IN CENTS	(2) PRICE RECEIVED FOR BUTTER, IN CENTS	(3) SCORE OF BUTTER	(4) PERCENTAGE OVERRUN	(5) COST OF LABOR PER HOUR, IN CENTS	(6) HOURS OF LABOR TO MAKE 1000 POUNDS OF BUTTER	(7) POUNDS OF BUTTER MADE IN 1919, IN THOUSANDS	(8) BUILDING COST PER 1000 POUNDS OF BUTTER, IN CENTS	(9) EQUIPMENT COST PER 1000 POUNDS OF BUTTER, IN CENTS
1. Price paid for fat, in cents.....		0 743±0 03421	0 631±0 04596	0 196±0 07343	0 008±0 07636	−0 536±0 05442	0 407±0 06371	−0 228±0 07239	−0 233±0 07222
2. Price received for butter, in cents . .			0 600±0 04887	0 101±0 07559	0 068±0 07601	−0 343±0 06738	−0 104±0 07554	−0 129±0 07509	−0.153±0 07458
3. Score of butter ...				−0 016±0 07635	0 123±0 07521	−0 378±0 06543	0 291±0 06990	−0 053±0 07615	0 103±0 07555
4. Percentage overrun					0 049±0 07618	−0 037±0 07626	0 143±0 07480	−0 110±0 07544	−0 050±0 07617
5. Cost of labor per hour, in cents.... .						−0 370±0 06591	−0 476±0 05906	−0 104±0 07630	0 034±0 07628
6. Hours of labor to make 1000 pounds of butter							−0 436±0 06185	0 418±0 07066	0.406±0 06378
7. Pounds of butter made in 1919, in thousands.........								−0 309±0 06907	0 246±0 07174
8. Building cost per 1000 pounds of butter, in cents ..									−0 241±0 07193
9. Equipment cost per 1000 pounds of butter, in cents ..									

When studying this table the reader should observe that the correlation is between the various readings on a line with the one at the head of the line. The four most significant columns are 2, 3, 6, and 7. There are, however, a few important correlations in columns 8 and 9.

shows a coefficient of only 0.196, in relation to this factor, which indicates that overrun in these creameries is not nearly so important a factor in affecting the price of butterfat as is the price of butter. There is one consideration in evaluating the significance of overrun, however, that should not be overlooked. If there was a greater variation in the percentage of overrun, the correlation would be higher. If the variations in overrun were from 10 to 24 per cent, the higher figures would have a greater bearing on the price of the butterfat than when the range is only from 18 to 26 per cent. Inasmuch as overrun is more uniform than a few of the other factors, it is of less importance.

The "price received for butter," "score of butter," "hours of labor to make 1000 pounds of butter," and "pounds of butter made in 1919, in thousands," have more to do with the price that the farmer receives for butterfat than do the other factors. It is also noticeable that "score of butter" has greater significance in relationship to "price received for butter" than does any other factor. Volume, or "pounds of butter made in 1919, in thousands," has a very low correlation with "price received for butter," whereas it was very significant when compared with the "price paid for fat."

About one-half of the correlations are positive and the other half are negative. However, if the correlation is 0.2 or 0.3 or more, it is significant regardless of whether it is positive or negative. The results of positive or negative correlations may be interpreted as follows. The coefficient of correlation of "price received for butter" and "price paid for fat" is 0.743, which means that the creamery which obtained a high price for its butter, paid a high price for the butterfat. Again, the correlation of "pounds of butter made in 1919, in thousands," to "cost of labor per hour, in cents," is –0.476, which shows that as the volume of butter manufactured declines, the cost of labor increases. It also shows a very significant relationship.

The "hours of labor to make 1000 pounds of butter" is highly correlated with the other items. On the other hand, the "cost of labor per hour, in cents," is of little importance. The probable explanation for so little correlation between these last two items is the same as in the case of the overrun. The cost of labor per hour is about the same throughout the state, consequently the low correlation. But there is a wide range of hours in "hours of labor to make 1000 pounds of butter," and therefore a significant correlation would be expected.

Under "building cost per 1000 pounds of butter, in cents," and "equipment cost per 1000 pounds of butter, in cents," the correlation coefficients are low with the exception of the correlations existing between them and "hours of labor to make 1000 pounds of butter." This indicates that as the cost of the building and that of the equipment go up, the hours of labor increase. A wide range in these two factors may be observed in table 3, which may account in part for the high coefficients. Another reason for high correlation coefficients may be in the large investments in building and equipment in creameries where the plant was not operated at full capacity.

MULTIPLE CORRELATION

A multiple correlation was calculated for the following four variables: "Price paid for fat in cents," "price received for butter in cents," "score of butter," and "hours of labor to make 1000 pounds of butter."

$$R_{1.236} = 0.82$$

It is apparent that the multiple correlation between these most significant variables is higher than any of the gross correlations. This indicates that the combined effect of the three independent variables is greater than any one.

It seems apparent that the amount of labor which goes into butter, and the volume of output, together with quality (which is measured by the score and reflected by the price of the butter), are the important considerations in making butter.

TABLE 3

Original data for nine variables for 78 Minnesota creameries, 1919

NUMBER OF CREAMERY	PRICE PAID FOR FAT, IN CENTS	PRICE RECEIVED FOR BUTTER, IN CENTS	SCORE OF BUTTER	PERCENTAGE OVER-RUN	COST OF LABOR PER HOUR, IN CENTS	HOURS OF LABOR TO MAKE 1000 POUNDS OF BUTTER	POUNDS OF BUTTER MADE IN 1919, IN THOUSANDS	BUILDING COST PER 1000 POUNDS OF BUTTER, IN CENTS	EQUIPMENT COST PER 1000 POUNDS OF BUTTER, IN CENTS
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
1	69.5	58.7	92	20.6	40.3	23.8	238	354	284
3	63.3	55.1	91	24.2	37.2	29.9	140	590	513
4	67.4	55.1	91	24.0	40.2	39.1	282	770	325
6	69.5	59.5	94	23.7	32.8	24.3	768	448	237
7	63.7	55.0	89	26.3	57.9	23.7	196	369	319
8	70.3	58.4	92	25.5	30.7	37.2	222	575	317
10	56.0	52.1	88	25.0	52.3	69.6	41	669	82
11	67.9	59.9	92	21.9	34.6	39.3	283	477	639
12	67.5	57.2	92	22.9	58.9	34.7	113	990	420
13	67.2	58.9	93	23.6	50.0	29.8	256	826	316
14	62.3	56.1	92	20.4	46.1	47.7	131	1780	807
15	68.5	58.5	93	26.0	64.7	28.2	225	776	483
16	68.0	58.7	92	26.4	46.8	34.0	170	1144	356
17	70.3	61.1	92	23.0	80.9	27.3	98	924	298
18	67.8	61.5	91	23.5	31.1	39.3	184	364	301
19	66.7	57.1	92	22.2	34.4	39.1	194	446	249
20	66.5	59.0	92	25.5	50.0	28.8	135	312	415
21	66.1	57.0	93	24.0	58.9	21.7	200	692	410
22	64.1	56.0	92	23.8	30.0	61.0	60	470	693
23	66.3	57.0	90	23.3	52.7	19.5	199	141	134
25	63.7	57.3	93	20.0	70.4	16.1	195	330	459
26	64.0	55.0	89	23.0	35.9	44.0	117	347	314
28	56.0	54.5	90	22.0	43.2	54.7	160	760	145
29	56.9	51.8	91	25.6	42.2	36.5	164	487	670
32	59.5	54.7	89	24.3	46.4	42.7	86	887	917
33	69.3	56.9	91	27.5	28.9	19.5	444	208	214
34	65.7	55.3	91	26.8	67.3	24.7	96	879	445
35	69.8	59.8	91.5	27.6	43.4	47.7	97	442	386
36	67.7	56.3	91.5	26.0	53.9	24.6	217	396	328
37	67.3	57.6	91	24.3	31.6	33.0	294	508	205
38	61.2	58.4	92	24.8	60.3	52.2	125	788	843
39	62.0	54.5	91	25.0	39.5	30.7	145	297	344
40	64.4	57.1	91.5	21.7	43.0	29.9	134	666	466
41	63.0	54.4	90	24.2	28.4	51.0	167	194	322
42	65.4	56.3	91.5	21.6	47.2	22.7	347	328	330
43	66.5	55.8	90	26.0	45.4	20.4	545	160	140
44	64.9	54.9	92	24.6	39.8	22.6	541	174	166
45	63.0	54.7	90	23.0	60.1	20.3	131	349	463

TABLE 3—*Continued*

NUMBER OF CREAMERY	PRICE PAID FOR FAT, IN CENTS	PRICE RECEIVED FOR BUTTER, IN CENTS	SCORE OF BUTTER	PERCENTAGE OVER-RUN	COST OF LABOR PER HOUR, IN CENTS	HOURS OF LABOR TO MAKE 1000 POUNDS OF BUTTER	POUNDS OF BUTTER MADE IN 1919, IN THOUSANDS	BUILDING COST PER 1000 POUNDS OF BUTTER, IN CENTS	EQUIPMENT COST PER 1000 POUNDS OF BUTTER, IN CENTS
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
46	63.7	56.6	89	25.2	42 5	37.9	492	460	384
47	64.2	55.1	91	24.3	40.1	32.3	198	219	335
48	62.0	55.3	91	23.1	51.4	34.0	106	1276	528
49	56.0	53.0	90	26.0	39.2	85.2	77	657	521
50	58.0	53.5	89	18.0	35.5	38.9	176	330	292
51	59.6	54.2	91	24.6	49 5	30.3	120	1029	380
52	67.8	58.5	92	23.5	27.6	40.0	209	433	320
53	65.8	55.6	91	24.5	26.2	46.9	209	530	469
54	67.1	55.8	92	24 7	44.7	26.8	246	428	425
55	68 0	57 1	92	24.0	49.7	17.8	443	454	349
56	67.0	55.2	91	24.0	41.1	33.0	197	876	588
57	68 2	58.8	93	25.2	38.7	23 1	464	543	456
58	68 7	57.3	93	23.2	42.5	26.5	559	252	290
59	64.3	56.9	92 5	25.0	42.7	27.8	256	538	464
60	58.1	55 2	90	22.7	44.3	72 1	62	1453	778
61	66.2	57.1	93	23 1	53.5	22.4	31	258	247
62	65.5	56.5	91.5	26.8	42.9	26.2	288	613	296
63	57 4	54.1	89	19 9	44 4	42 9	167	774	293
65	60.0	55.0	88	24 8	28.8	75.0	110	1515	621
68	64.1	55.4	91	22 5	28.5	34.0	236	928	492
69	68 5	56 9	92	22 7	40 2	23.1	263	319	694
70	63.0	54.5	91.5	24.0	57 0	24.2	147	286	659
71	68.8	54.3	92	22.8	38.3	78.5	136	1104	899
72	58.0	55.7	93	23 3	35.9	68.4	178	472	757
78	61.2	57.6	88	25.4	41.3	48.7	243	334	247
79	64 5	54 9	88	23 1	36 2	27.4	265	215	222
80	57.0	53.1	87	23 0	35.6	60.7	107	998	496
81	66.0	56.9	92	24.2	55 5	21 9	886	525	274
82	69.2	59.2	93	23 8	48.5	35.2	273	700	372
83	64.1	54.3	91	27.0	52 4	34.9	128	652	663
87	64.2	56.0	91	24 0	70.0	16.6	211	398	532
91	61.1	52.3	90	19.1	47.1	34 8	200	257	445
92	58.9	54.8	91	22.1	44.6	40.9	138	948	581
93	63.5	53.0	91	22.3	41.3	34.8	221	1400	292
94	69.3	58.6	94	24.0	55 3	20.2	341	487	297
96	68.8	56.3	92	24.7	39.7	25.4	482	799	399
97	68.5	58.8	94	23.2	37.4	33.0	200	603	478
98	67.8	58.2	92	22.1	44.0	26.8	207	833	331
99	70.2	59.1	94	22.8	27.1	36.8	339	573	376
101	67.5	58.2	91	25.5	43.3	27.9	206	292	516

TABLE 3—*Concluded*

For the student who desires to pursue the study further. The original data is presented in table 3. The sums of the nine variables, the sums of their squares, and the sums of their products are as follows:

ΣX_1 = 5051.00
ΣX_2 = 4396 10
ΣX_3 = 7116 50
ΣX_4 = 1858 00
ΣX_5 = 3461 90
ΣX_6 = 2776 50
ΣX_7 = 17957 00
ΣX_8 = 47078 00
ΣX_9 = 32813 00

ΣX_3X_4 = 169518 50
ΣX_3X_5 = 316013.35
ΣX_3X_6 = 252645 85
ΣX_3X_7 = 164356.00
ΣX_3X_8 = 4293134.50
ΣX_3X_9 = 2995947 50

ΣX_7X_8 = 9594909 00
ΣX_7X_9 = 6760306 00

M.X_1 = 64 7564
M X_2 = 56.3602
M.X_3 = 91 2371
M X_4 = 23.8205
M X_5 = 44 3833
M X_6 = 35 5961
M.X_7 = 230 2179
M.X_8 = 603 5641
M X_9 = 420.6794

M X_3X_4 = 2173 3141
M X_3X_5 = 4051 4532
M X_3X_6 = 3239 0416
M X_3X_7 = 21071 2820
M X_3X_8 = 55040 1860
M X_3X_9 = 38409 5833

M.X_7X_8 =123011 6500
M X_7X_9 = 86670 5897

ΣX^2_1 = 328274 24
ΣX^2_2 = 248099 01
ΣX^2_3 = 649464 75
ΣX^2_4 = 44523 10
ΣX^2_5 = 163061 95
ΣX^2_6 = 116688.53
ΣX^2_7 = 59455 82
ΣX^2_8 = 373538 68
ΣX^2_9 = 16354309 00

ΣX_4X_5 = 82542 30
ΣX_4X_6 = 66058.08
ΣX_4X_7 = 430868 26
ΣX_4X_8 = 1116102 90
ΣX_4X_9 = 780318 60

ΣX_8X_9 =21846095 00

M X^2_1 = 4208 6441
M X^2_2 = 3180 7565
M X^2_3 = 8326 4711
M X^2_4 = 570 8089
M X^2_5 = 2090 5378
M X^2_6 = 1496.0068
M.X^2_7 = 762 2541
M.X^2_8 = 4788.9574
M X^2_9 =209670 6282

M X_4X_5 = 1058 2346
M.X_4X_6 = 846 8984
M X_4X_7 = 5523 9520
M X_4X_8 = 14309 0110
M X_4X_9 = 10004 0846

M X_8X_9 =280078 1412

ΣX_1X_2 = 285143 81
ΣX_1X_4 = 461125 75
ΣX_1X_4 = 120423 81
ΣX_1X_5 = 224204 00
ΣX_1X_6 = 177321 10
ΣX_1X_7 = 1181768 10
ΣX_1X_8 = 3025057.00
ΣX_1X_9 = 2111972 90

ΣX_5X_6 = 118419 20
ΣX_5X_7 = 734699 00
ΣX_5X_8 = 2059378 00
ΣX_5X_9 = 1461654 60

M X_1X_2 = 3655 6898
M X_1X_3 = 5911 8686
M X_1X_4 = 1543 9591
M.X_1X_5 = 2874 4102
M X_1X_6 = 2273 3474
M X_1X_7 = 15150 8730
M X_1X_8 = 38782 7820
M.X_1X_9 = 27076 5756

M X_5X_6 = 1518 1948
M X_5X_7 = 9419 2179
M.X_5X_8 = 26402 2820
M X_5X_9 = 18739 1615

ΣX_2X_3 = 401231 90
ΣX_2X_4 = 104749 04
ΣX_2X_5 = 195232.84
ΣX_2X_6 = 155642 54
ΣX_2X_7 = 100844.15
ΣX_2X_8 = 2646253 30
ΣX_2X_9 = 1814878 50

ΣX_6X_7 = 560783 90
ΣX_6X_8 = 1842981 20
ΣX_6X_9 = 1254757.60

M X_2X_3 = 5143 9987
M X_2X_4 = 1342 9364
M X_2X_5 = 2502 9851
M X_2X_6 = 1995 4172
M X_2X_7 = 1292 8737
M X_2X_8 = 33926 3240
M X_2X_9 = 23652 2884

M X_6X_7 = 7189 5372
M X_6X_8 = 23627.964
M X_6X_9 = 16086 6358

FURTHER INVESTIGATIONS WITH ADULTERATING SULPHURIC ACID SO AS TO INCREASE BABCOCK TEST READING*

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Two years ago the writer[1] reported on the possibility of adulterating sulfuric acid so as to increase the reading of the Babcock test in which such acid was used. That report was on a temporary emulsion of a fat solvent saturated with butterfat and thoroughly shaken with acid. This adulteration did not materially affect the appearance of the acid, but on standing for more than an hour, the fat saturated solvent would separate out and accumulate on top of the acid, in which condition duplicate tests would not check and consequently adulteration would be suspected.

Since that report, the writer has been able to secure an acid emulsion that is stable and that will increase the Babcock reading when such acid is used. One sample of such an emulsion made up on December 15 has stood without agitation, and on May 5 shows no signs of separation. This sample was made up of 400 cc. sulfuric acid, specific gravity 1.83, 16 cc. fat saturated benzine, and 6 cc. water soap solution (Palmolive china soap, 1 part, and water, 2 parts). As the soap water solution is not miscible with the benzine, it was added first to the cold acid and shaken when the saturated fat benzine solution was added and thoroughly shaken.

This acid was used in performing a Babcock test the following day without being agitated, when it gave an increased reading of 0.8. It was again used without agitation on February 15, when the necessary sample was carefully drawn by means of a

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[1] W. E. Petersen, JOURNAL OF DAIRY SCIENCE, vii, no. 4 (1924).

pipette from about the center, with the same results. Further tests on April 15 gave identical results.

Centrifuging this acid in an 18-inch centrifuge at about 10,000 revolutions per minute for ten minutes failed to cause separation. Examination under an oil emersion lens of 1200 magnifications failed to reveal any particles, indicating that in all probability the dispersion is colloidal and that the soap acts as a stabilizer.

The appearance of the adulterated acid would not arouse suspicion nor would its reactions with milk, in performing the Babcock test, as it behaves like normal acid. Separation of the adulterant is effected, however, by the addition of equal parts of water and centrifuging in usual Babcock test bottles in a Babcock centrifuge at the normal speeds for milking testing, by which means adulteration can be detected.

THE EFFECT OF THE PASTEURIZATION TEMPERATURE ON INDIVIDUAL GERMS FOUND IN MILK*

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Pasteurization, since its introduction into the dairy industry, has received much study. The primary reason for introducing this process into the dairy business was to protect the public from disease germs carried by milk and it is along this line that most of the study has been directed. Pasteurization also prolongs the keeping quality of the milk when it is properly done. It is stated that about 99 per cent reduction of the bacterial milk flora is obtained by this process. Ayers and Johnson (1) have published evidence showing that when milk is highly contaminated with bacteria a ninety-nine per cent reduction is secured, but when the milk is not so highly contaminated the percentage of reduction does not reach this figure.

The effect of pasteurization on the individual organism found in milk has not received much study. Ayers and Johnson (3) found that of twenty-two typical streptococci one survived a temperature of 62.8°C. for thirty minutes, and of 117 atypical streptococci, 38.46 per cent survived this temperature. The same authors (2) found twelve colon bacilli which resisted a temperature of 62.8°C. Barber (4), studying the effect of temperature on the rate of multiplication of a colon bacterium, found that it reached its maximum rate of division at 37°C. and that this rate fell off at 45°C. and ceased at 49°C. Tanner and Dubois (6) concluded from their work "that the members of the colon-typhoid group in milk in the number to which they would occur are destroyed by heating for thirty minutes at 60°C."

The purpose of this study was to determine the effect of pasteurization on pure cultures of bacteria found in milk. The

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† **This short paper is to be considered as a preliminary report.**

work was commenced June 15, 1925, and was concluded August 8, 1925. During this period seventy different types of bacteria were collected. These organisms were taken from platings made from unpasteurized milk, from rinsings made from dairy utensils and from the interior of the cow's udder. The organisms to be pasteurized were transferred to test tubes containing 5 cc. of sterile milk. The inoculated test tubes were incubated at 37°C. from two to five hours. Pasteurization was carried on in a copper jacketed water bath at a temperature of 62.5°C.

Just before pasteurization the milk was plated and then put into a water bath and pasteurized at a temperature of 62.5°C. for thirty-five minutes. The five minutes over the usual pasteurization time was to allow the milk to reach the above temperature. The milk was again plated immediately after pasteurization. The plates were incubated for ninety-six hours at room temperature and twenty-four hours at 37°C. If the plates made from pasteurized milk were not sterile, the organism was again inoculated into sterile milk, incubated and repasteurized using, instead of a test tube, a Sternberg bulb of about 5 cc. capacity. In some instances, it was found that milk would not be sterile when pasteurized in a test tube, but would be sterile when pasteurized in a Sternburg bulb. After the milk was pasteurized and plated, it was incubated for several days to observe whether the destruction of the germs was complete. An effort was made to not allow the germs to grow to such a number that their by-products would be a factor in their destruction during pasteurization.

All the organisms reported in table 1 produced acid in nutrient broth containing dextrose, and most of them produced acid in nutrient broth containing sucrose and lactose. None produced gas excepting the aerogenes like organism and the colon organisms.

There were forty-seven non-spore-forming organisms. Of these forty-seven, only two survived pasteurization. The two resisting organisms are 43 and 44. No. 44 is similar to, if not the same as, the organism reported by Russel and Hasting (5) several years ago to withstand a temperature of 76°C. It is a lemon color micrococcus. It produces acid in nutrient broth and grows rapidly in milk. Russel and Hastings report that their organism

TABLE 1

The effect of pasteurization on germs found in milk and dairy utensils

NUMBER OF ORGANISMS	UNPASTEURIZED	PASTEURIZED	REMARKS
Non-spore formers			
1	36,000,000	Sterile	
2	3,420,000	Sterile	Ropy
3	1,790,000	Sterile	
4	7,660,000	Sterile	
5	16,140,000	Sterile	
6	32,400,000	Sterile	
7	43,200,000	Sterile	
8	115,000	Sterile	
9	7,300,000	Sterile	
10	166,800,000	Sterile	
11	61,200,000	Sterile	
12	28,900,000	Sterile	
13	1,620,000	Sterile	
14	1,020,000	Sterile	
15	651,000	Sterile	
16	54,600,000	Sterile	
17	11,000	Sterile	
18	23,500,000	Sterile	
19	27,000,000	Sterile	
20	1,185,000	Sterile	
21	59,400,000	Sterile	
22	114,000,000	Sterile	
23	14,500,000	Sterile	
24	330,000,000	Sterile	
25	109,200,000	Sterile	
26	112,900,000	Sterile	
27	2,100,000	Sterile	
28	114,000,000	Sterile	Gas forming. Aerogenes-like
29	78,000,000	Sterile	Somewhat similar to 28
30	5,100,000	Sterile	
31	21,800,000	Sterile	
32	1,500,000	Sterile	
33	3,400,000	Sterile	White colony, coccus, from U
34	6,500,000	Sterile	Yellow colony, coccus, from U
35	4,700,000	Sterile	Pink colony, coccus, from U
36	1,500,000	Sterile	
37	6,100,000	Sterile	Colon, human
38	20,000	Sterile	
39	27,000	Sterile	
41	30,600,000	Sterile	
42	40,200,000	Sterile	

TABLE 1—*Continued*

NUMBER OF ORGANISMS	UNPASTEURIZED	PASTEURIZED	REMARKS
	Non-spore formers—Continued		
43	36,000,000	1,020,000	
44	5,040,000	134,000	Micrococcus lemon color
45	10,000,000	Sterile	
46	15,000,000	Sterile	Streptococcus
47	2,100,000	Sterile	Aerogenes with reddish color
	Spore formers		
48	2,850,000 600,000 4,600,000 2,250,000 4,800,000 2,500,000	3,060,000 460,000 2,390,000 5,050,000 9,600,000 4,040,000	Brownish colony, rod forms spores so rapidly that it is hard to find vegetative organism
49	4,120,000	30,000	
50	640,000	33,000	
51	352,000	25,900	
52	165,000	96,000	
53	1,080,000	132,000	
54	6,700,000	300	Spores not numerous
55	13,000,000	2	Spores not numerous
56	84,000,000	500	Spores not numerous
57	1,200,000	100	Spores not numerous
58	21,000,000	10,810,000	
59	3,480,000	2,160,000	
60	800,000	780,000	
61	4,400,000	1,120,000	
62	1,600,000	149,000	
63	5,900,000	4,540	
64	180,000,000	7,200	
65	2,800,000	9,500	
66	72,000,000	1,000	
67	5,040,000	134,000	
68	1,560,000	1,140,000	
69	9,300,000	1,000	
70	14,600,000	1,020,000	

did not ferment dextrose, sucrose or lactose. No. 43 was not picked from the plates this year, but was secured from pasteurized milk two years ago and kept in the laboratory. At the time when it was found, it was causing counts of a million or more in pasteurized milk.

Most of the spore forming organisms reported here first coagulate milk and then digest it. But some of them digest the milk without coagulating it. No thermophils were found during the progress of this work.

TABLE 2

Effect of pasteurization on named organisms

NAME OF ORGANISMS	UNPASTEURIZED	PASTEURIZED
Bacillus viscosus	10,900,000	Sterile
Bacillus subtilus	5,200,000	32,100
Bacillus ramosus	2,400,000	24,000
Bacillus buytricus	4,200,000	266,000
Bacillus Glaligu.	14,900,000	980
Proteus vulgaris	84,000,000	Sterile
Erythrobacillus ptodiosus	700,000	Sterile
Bacterium paracoli	7,200,000	Sterile
Bacterium communior	26,600,000	Sterile
Bacterium coli	9,400,000	Sterile
Bacterium aerogenes	8,800,000	Sterile
Bacterium acid lacti	144,000,000	Sterile
Bacterium aerogenes	2,000,000	Sterile
Bacterium alcaligenes	2,000,000	Sterile
Bacterium aerogenes soil	44,000,000	Sterile
Bacterium coli (bovine)	13,000,000	Sterile
Pseudomonus pyocyaneus	12,300,000	Sterile
Micrococcus albus	2,100,000	Sterile
Micrococcus Aurantiaca	144,000,000	Sterile
Staphylococcus aureus	7,900,000	Sterile
Micrococcus aureus	780,000	Sterile
Sarcina ventriculi	420,000	Sterile
Sarcina lutia	197,000	53,000
Micrococcus flavus	7,000,000	Sterile
Lactobacillus casei (epilson)	1,140,000	Sterile
Lactobacillus casei (alpha)	3,500,000	Sterile

Note: The authors accepted the names of the above organisms as they were on the tubes when received and no effort was made to verify them.

There was no instance where a spore forming organism was entirely eliminated by pasteurization. In two instances a larger count was obtained in the pasteurized milk than in the unpasteurized milk. This increase in number is apparently due to the breaking up of clumps of spores, since neither of these two organisms grew at temperatures above 50°C.

There was no effort made to determine the names of the organisms used in obtaining the data in table 1. The only criterion used was that the colonies were visibly different. In table 2 are given data on pasteurization of named organisms.

The outstanding point in table 2 is the same as in table 1, namely, that nearly all non-spore forming organisms were destroyed by pasteurization. Sarcina lutia is the exception in this case.

It will also be noted that the three colon organisms used in this work did not survive pasteurization. This is of interest because of the discussion as to whether all colon bacteria are destroyed by pasteurization.

Since only few of the organisms found in milk have been used in this work, no definite conclusion can be stated. More organisms were not used simply because they did not occur on the plates. This limited number may have been due to the fact that during the period of this experiment the weather conditions remained practically constant.

But the data presented indicate that a large number of the organisms found in milk do not survive a temperature of 62.5°C. Yet, in pasteurized milk, high counts are continually being found and these high counts are mostly due to non-spore-forming organisms. The reason for this is a problem for further investigation.

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A STUDY OF METHODS FOR BACTERIAL ANALYSES OF MARKET MILK*

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Bacterial counts of milk made upon different kinds of media, vary considerably in number present per cubic centimeter. This is due, largely, to the composition of the media used. As one kind of medium is most suitable for the growth of certain types of bacteria, it will give higher counts than other media when that particular type of organism predominates. If another type of organism predominates in the milk, another medium may be expected to give the higher counts.

It occasionally happens that a sample of milk with a count of 100,000 bacteria per cubic centimeter upon one medium will have a count of only half that number upon another medium. With other samples of milk the reverse may happen. Usually, however, certain media tend to give the highest counts, while other media tend to give the lowest counts.

The usual reason for making bacteriologicaal counts upon milk is to get an idea of how carefully the milk has been handled up to the time that the sample was taken. If the bacteriological count is low, we assume that the milk has been handled in such a careful way that good keeping quality and reasonable safety are likely. If the count is very high, we suspect that the milk has been so carelessly handled that good keeping qualities cannot be expected and safety from disease producing organisms is in doubt.

As a member of the committee upon methods of bacterial analyses of milk and milk products appointed by the International Association of Dairy and Milk Inspectors, I undertook in 1921 to show the value of plate counts obtained upon four kinds of

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† Professor Cooledge died in May, 1925. This paper was nearly ready for publication and is presented with some slight changes designed to clarify certain points which seemed a little obscure.—*G. L. A. Ruehle.*

agar commonly used in milk work and to show the relation of the counts to the pH score (1).

TABLE 1

Samples of milk in group I

		pH SCORE	BACTO AGAR	MEAT EXTRACT	MEAT INFUSION	AGAR A
1	Farmer 12	50	50,000,000	60,000,000	22,000,000	67,000,000
2	Same; a cool day	45	9,600,000	1,000,000	2,300,000	1,700,000
3	Farmer 22	50	600,000	160,000	250,000	800,000
4	Same; a cool day	95	150,000	30,000	50,000	100,000
5	Farmer 8	45	600,000	650,000	400,000	700,000
6	Same; a cool day	55	60,000	80,000	40,000	10,000
7	Farmer 25	45	100,000		200,000	
8	Same; a cool day	90	80,000	50,000	40,000	10,000
9	Farmer 13	50	400,000	200,000		
10	Same; a cool day	95	110,000	120,000	60,000	100,000
11	Farmer 21	45	5,000,000	6,000,000	12,000,000	8,000,000
12	Same; a cool day	55	1,500,000	1,000,000	1,200,000	2,500,000
13	Farmer 36	50	75,000	10,000	40,000	60,000
14	Same; a cool day	70	50,000	30,000	30,000	30,000
15	Farmer 24	50	700,000	180,000	360,000	
16	Same; a cool day	80	130,000	180,000	90,000	60,000
17	Farmer 20	45	1,000,000	1,500,000		
18	Same; a cool day	55	130,000	100,000	40,000	40,000
19	Farmer 54	50	100,000	700,000	700,000	800,000
20	Same; a cool day	55	45,000	80,000	40,000	20,000
21	Farmer 1	80	30,000	7,000	80,000	110,000
22	Same; a cool day	95	150,000	80,000	20,000	10,000
23	Farmer 6	50	120,000	210,000	180,000	300,000
24	Same; a cool day	90	10,000	50,000	20,000	10,000
25	Farmer 11	45	1,000,000	1,000,000	500,000	1,000,000
26	Same; a cool day	55	220,000	100,000	90,000	100,000
27	Farmer 5	45	1,000,000	12,000,000	40,000,000	15,000,000
28	Same; a cool day	90	90,000	40,000	20,000	140,000
29	Farmer 51	45	6,000,000	2,400,000		
30	Same; a cool day	90	10,000	30,000	10,000	10,000
31	Farmer 90	40	500,000	450,000	500,000	800,000
32	Farmer 50	80	370,000	50,000	90,000	100,000
33	Farmer 16	40	5,000,000	3,600,000		6,000,000
34	Farmer 2	50	3,300,000	3,100,000	4,000,000	5,000,000
35	Farmer 14	50	3,200,000	3,600,000	5,000,000	
36	Farmer 10	85	200,000	100,000	140,000	140,000

Early results of this work have been included in the committee reports (2), but my work has been continued and extended.

The methods used in this work are the 1921 Standard Methods for Bacteriological Examination of Milk, published by the American Public Health Association. Bacto agar used in groups I and II is out of lot 10445 which was furnished by the Digestive Ferments Company to members of the committee. Other Bacto agar was out of regular stock. Milk-powder agar was the milk-powder agar A described by Ayers and Mudge (3).

Media were adjusted to pH 6.5 to 6.8 where necessary. It was not necessary to adjust the reaction of Bacto agar and agar A.

Group I includes 36 samples of milk, 18 of which were taken upon a warm day and tested at once. The other 18 samples were taken from the same patron's cans a few days later, but upon a very cool day.

Group II includes 48 samples of milk, 24 of which were plated upon the four kinds of media and the pH score determined. The samples were then incubated at room temperature for two hours and retested.

Group III includes samples of milk plated upon different media and incubated at 37°C. for one hour and retested.[1]

It is fair to assume that the samples of milk in groups II and III when plated the second time should be in poorer condition bacteriologically than when they were tested the first time. If this is true, the method which shows the samples in poorer condition most consistently when tested the second time would be the better method.

Comparing the tests and retests on all of the samples of milk in group II and III using both the pH method and counts in various media, the sensitiveness of the methods appears as follows:

	Per cent efficient
pH score	91.0
Bacto agar	66.6
Meat infusion agar	66 6
Milk powder agar A	63.3
Beef extract agar	60.0

Greater sensitiveness of methods would be expected if the samples have a high initial bacterial count. Methods which show

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[1] The detailed data for this could not be found.—*G. L. A. Ruehle.*

TABLE 2

Samples of milk in group II

		pH SCORE	BACTO AGAR	MEAT EXTRACT AGAR	MEAT INFUSION AGAR	AGAR "A"					LACTOSE INFUSION AGAR	LACTOSE EXTRACT AGAR
						Bacteria per cubic centimeter	Strong acid	Weak acid	Pepton-izers	Alk. and inert		
51	55	100	3,000	2,000	1,000	1,000				1,000		
52	55*	100	4,000	3,000	3,000	1,000				1,000		
53	6	100	5,000	7,000	8,000	8,000			1,000	7,000		
54	6*	95	7,000	1,000	1,000	2,000		1,000		1,000		
55	14	100	5,000	4,000	4,000	2,000				2,000		
56	14*	95	6,000	1,000	6,000	7,000				7,000		
57	21B	100	12,000	17,000	12,000	25,000		3,000	6,000	16,000		
58	21B*	85	14,000	9,000	7,000	16,000	2,000	4,000	1,000	9,000		
59	Raw, East Lansing Supply	100	9,000	10,000	15,000	15,000		10,000		5,000		
60	Raw, East Lansing Supply*	75	4,000	2,000	8,000	9,000		3,000		6,000		
61	19	95	10,000	10,000	10,000	10.000				10,000	10,000	10,000
62	19†	95	20,000	30,000	10,000	20,000				10,000	20,000	10,000
63	36A	95	10,000	10.000	10,000	10,000				10,000		
64	36A†	90+	10,000	20,000	30,000	40.000				40,000		
65	Pasteurized	95	10,000	10,000	10,000	90,000			10,000	80,000	20,000	10,000
66	Pasteurized†	90	20,000	50,000	10,000	40,000				40,000	20,000	10,000
76	13	95	20,000	20,000	30,000	10,000				10,000		
68	13†	90	10,000	10,000	10,000	40.000			10,000	30,000		
69	10	90	13,000	12,000	4,000	17,000			2,000		15,000	
70	10*	80	18,000	12,000	5,000	16,000	2,000			14,000		
71	24	80	20.000	10,000	30,000	20,000				20,000		
72	24†	80	10,000	30.000	100,000	40,000				40,000		
73	17	90	70,000	40,000	100,000	10,000				10,000	60,000	30,000

74	17†	75	60,000	20,000	20.000	40,000				40,000	50,000	90,000
75	50	85	60,000	80,000	90,000	110.000			20,000	90,000	50,000	120,000
76	50†	75	140,000	30,000	230,000	290,000				290,000	230,000	170,000
77	36B	85	12,000	10,000	12,000	19.000	6,000	12,000		1,000		
78	36B*	60	17,000	5,000	10,000	21,000		8,000		13,000		
79	23A	80	80,000	10,000	40,000	20,000				20,000		
80	23A†	80	60,000	30.000	60,000	60,000				6,000		
81	11	80	14,000	27,000	25,000	25,000	1,000	3,000	3,000	15,000		
82	11*	70	12,000	16.000	3,000	19.000		1,000	2.000	16.000		
83	23B	80	10,000	8.000	3,000	9,000				9,000		
84	23B*	55	18,000	3,000	7,000	7,000				7,000		
85	21A	75	160,000	250.000	270.000	900,000	50,000	50,000	10,000	790.000		
86	21A†	55	230,000	300,000	650,000	800,000	30,000			770,000		
87	8B	70	35,000	16,000	16,000	28,000	4,000	10,000	6,000	8,000		
88	8B*	50	150,000	117,000	580.000	250,000	20,000	100,000	5,000	125,000		
89	8A	65	20,000	10,000	20,000	50,000				50,000	90,000	10,000
90	8A†	55	80,000	30.000	30.000	50,000			20.000	30,000	400,000	70,000
91	16	55	2,100.000	1,200,000	700,000	900,000				900,000	640,000	1,000,000
97	16†	50	3,500,00	4,000,000	5,000,000	5,500,000				5,500,000	4,500,000	4,600,000
93	20	55	90,000	50,000	90,000	80.000				80,000	80,000	
94	20†	50	60,000	70.000	110,000	40,000				40,000		
95	2A	50	1,800,000	1,400,000	1,300,000	1,400,000	150,000	50,000	500,000	700,000		
96	2A†	45	2,700.000	3,400,000	2,600,000	2,100,000	120,000		1,500,000	480,000		
97	2B	45	900,000	254,000	1,200,000	1,030,000	130,000	210,000	60.000	630.000		
98	2B*	45–	1,140,000	950,000	5,500,000	4,500,000	100,000	160,000	130,000	4,101,000		

* Two hours at room temperature.

† Two hours at room temperature; twenty-two hours in ice box.

poorer condition upon retest of samples with low initial counts should be the more valuable.

If only those samples of milk having a retest average count of under 25,000 bacteria per cubic centimeter are considered, the efficiency of the various methods and media is as follows:

	Per cent efficient
pH score	84.6
Bacto agar	61.5
Infusion agar	30.0
Agar A	30.0
Extract agar	23.0

Considering only those samples having a retest average count between twenty-five thousand and 100,000, the results are as follows:

	Per cent efficient
pH score	85.7
Infusion agar	85.7
Agar A	85.7
Extract agar	71.4
Bacto agar	28.5

The samples of milk having retest counts between 100,000 and 1,000,000 bacteria per cubic centimeter give the following results:

	Per cent efficient
pH score	100
Infusion agar	100
Bacto agar	80
Extract agar	80
Milk powder agar A	60

The samples of milk with retest counts of over 1,000,000 give results as follows:

	Per cent efficient
pH score	100
Bacto agar	100
Extract agar	100
Agar A	100
Infusion agar	87.5

The pH score of the samples of milk after incubation was at times the same as before, but it never indicated that the milk was in better condition after the samples had been subjected to the short incubation period and retested. In three instances the pH score indicates that the milk was in no worse condition after incubation that it was before. These were samples of milk which were usually good by this method when tested the first time.

In certain instances the samples of milk tested with the different kinds of media before and after incubation gave results which indicated that they were as good after the incubation as before. The samples may be classified as shown in table 3.

It seems that after the treatment which the above samples received, efficient methods should show the samples in worse condition upon retest. A few of the samples which were in very good condition at the start might show no change upon retest, but certainly a reliable method should not indicate that a third of the samples were in an improved condition.

TABLE 3

Condition of milk samples upon retest after short incubation period

HOW TESTED	POORER	NO CHANGE	BETTER
Meat extract agar	20	1	12
Milk powder agar A	20	2	11
Bacto agar	21	1	11
Meat infusion agar	22	2	9
pH score	29	3	0

Many investigations have been made to determine the most suitable medium for milk work. It has usually been concluded in these studies that the method giving the highest counts is most efficient. It is argued that the medium giving the highest counts comes closest to giving counts which represent all of the bacteria present in the milk. There is a failure to agree upon the medium giving the highest counts. The reasons for this failure are considered below.

A tabulation of the samples of milk in groups I, II, and III, with average bacterial counts below 10,000 per cubic centimeter when plated upon four kinds of media shows that Bacto agar gives the highest counts and the milk powder agar A is next, with meat extract agar the lowest. When samples with 10,000 to 25,000 bacteria were studied the counts upon milk powder agar A are the highest, with meat extract agar next, and meat infusion agar giving the lowest counts.

If a preponderance of samples is present with low counts one investigator might be expected to find one medium giving the highest counts while another investigator studying a poorer grade of milk would find that another medium would give the highest counts.

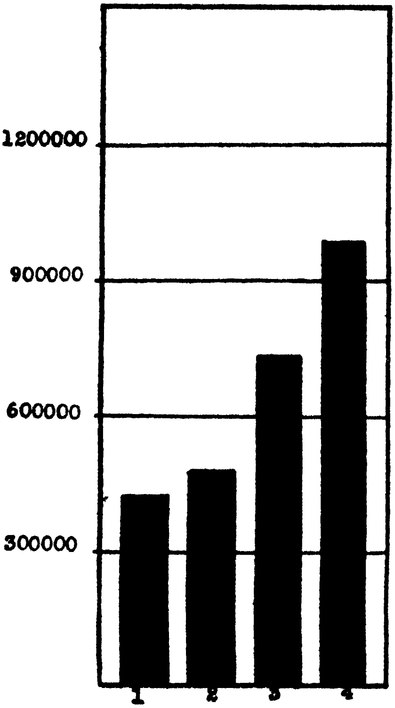

Fig. 1

The 102 samples of milk in groups I, II, and III gave average counts upon the four kinds of agar as shown in figure 1.

When these samples were divided into groups depending upon the average count upon the four kinds of agar the medium giving the highest counts for the different groups is indicated in table 4.

The average bacterial counts of samples tabulated in groups I, II and III when arranged according to their pH scores indicates that while the pH score does not check with the bacterial count in many cases, it does check in a general way. Samples scoring 75, 70, 65, 60, and 55, because of the small numbers of samples in the various groups, do not check when taken separately but do check when arranged together as shown in table 5.

TABLE 4

Medium giving highest counts for the different groups

NUMBER OF BACTERIA PER CUBIC CENTIMETER AVERAGE WITH 4 KINDS OF AGAR	HIGHEST AVERAGE WITH	NEXT HIGHEST WITH	NEXT LOWEST WITH	LOWEST AVERAGE WITH
Under 10,000	Bacto agar	Agar A	Infusion agar	Extract agar
10,000– 25,000	Agar A	Extract agar	Bacto agar	Infusion agar
25,000– 50,000	Bacto agar	Agar A	Infusion agar	Extract agar
50,000– 100,000	Bacto agar	Agar A	Infusion agar	Extract agar
100,000– 200,000	Bacto agar	Agar A	Infusion agar	Extract agar
200,000–1,000,000	Agar A	Infusion agar	Bacto agar	Extract agar
Over 1,000,000	Bacto agar	Agar A.	Extract agar	Infusion agar

TABLE 5

Showing the relation of average bacteriological counts and average of pH scores

NUMBER OF SAMPLES	WITH A pH SCORE OF	AVERAGE BACTERIAL COUNT ON FOUR KINDS OF MEDIA
7	100	10,300
10	95	34,200
10	90	44,600
4	85	63,500
10	80	92,800
8	75, 70	150,000
13	65, 60, 55	562,000
14	50	5,086,000
13	45	4,178,000
2	40	2,714,000

The samples of milk scoring 50, 45 and 40 show a decrease in bacterial count. This is probably due to the fact that some of the inert types find the increased activity of the acid bacteria unfavorable.

TABLE 6

Comparisons of the pH score, reductase grade and the bacterial plate count on 82 samples of milk

LABORATORY NUMBER	pH SCORE	REDUCTASE	BACTERIA PER CUBIC CENTIMETER BACTO AGAR, pH 6.8
1	90	Good	10,000
2	80	Good	55,000
3	80	Good	50,000
4	95	Good	10,000
5	80	Good	120,000
6	55	Fair	1,500,000
7	75	Fair	300,000
8	80	Good	160,000
9	95	Good	85,000
10	90	Good	80,000
11	95	Good	110,000
12	50	Good	3,000,000
13	95	Good	6,000
14	90	Good	20,000
15	75	Fair	6,000
16	95	Good	100,000
17	90	Good	25,000
18	80	Good	35,000
19	65	Good	40,000
20	95	Good	10,000
21	90	Good	24,000
22	85	Fair	220,000
23	80	Good	80,000
24	85	Good	30,000
25	75	Good	500,000
26	80	Good	30,000
27	95	Good	16,000
28	50	Bad	65,000,000
29	40	Very bad	110,000,000
30	45	Very bad	50,000,000
31*			
32	45	Very bad	160,000,000
33	35	Very bad	175,000,000
34	40	Very bad	90,000,000
35	45	Very bad	125,000,000
36	45	Very bad	70,000,000
37	50	Very bad	45,000,000
38	50	Very bad	65,000,000
39	35	Very bad	180,000,000
40	55	Fair	5,000,000
41	55	Fair	6,000,000
42	45	Fair	175,000,000
43	55	Bad	65,000,000
44	55	Very bad	2,000,000
45	45	Bad	60,000,000
46	35	Very bad	140,000,000
47	75	Fair	1,800,000
48	55	Bad	75,000,000
49	45	Very bad	120,000,000
50	45	Bad	110,000,000
51	45	Bad	45,000,000
52	45	Very bad	160,000,000
53	45	Bad	80,000,000
54	50	Bad	30,000,000
55	35	Very bad	50,000,000
56	50	Fair	4,000,000
57	45	Very bad	840,000
58	55	Fair	35,000
59	55	Fair	220,000
60	55	Fair	420,000
61	55	Fair	9,000,000
62	50	Fair	84,000,000
63	55	Fair	42,000
64	55	Fair	660,000
65	55	Fair	460,000
66	35	Very bad	84,000,000
67	50	Fair	280,000
68	50	Bad	1,200,000
69	55	Fair	30,000,000
70	50	Bad	1,500,000
71	45	Bad	700,000
72	50	Bad	850,000
73	90	Good	300,000
74	50	Fair	100,000
75	50	Fair	980,000
76	45	Bad	2,000,000
77	45	Bad	40,000,000
78	50	Bad	2,500,000
79	85	Fair	40,000
80	55	Fair	700,000
81	55	Fair	500,000
82	55	Fair	250,000

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* Plate contaminated.

This table does not include the nine samples of milk in group III which were incubated at 37°F. or the two samples of pasteurized milk in group I.

A matter, closely related to the above, is some comparisons which were made between the pH method, the reductase test, and the official plate count of bacteria in milk. In this work there are 82 samples of milk which were tested by the three methods. The plating technique was that given in the Fourth Edition of "Standard Methods of Milk Analysis," published by the American Public Health Association. The reductase test was that given as a provisional method in the same publication. Methylene blue tablets sold by the National Aniline and Chemical Company were used. Correction as to the strength of the methylene blue solution was noted.

The results are recorded in table 6.

A study of the data reveals the fact that the pH method correlates as well with the average bacterial count as does the reductase test. The following parallel comparisons will show this.

Reductase ratings:	*Average bacteria Per centimeter*
Good (24 samples)	204,000
Fair (24 samples)	6,104,000
Bad (15 samples)	38,583,000
Very bad (18 samples)	100,000,000
pH score ratings:	
Good	68,000
Fair	529,000
Bad	19,000,000
Very bad	90,000,000

Looking at the individual ratings the following facts are brought out:

2 samples classified as good by reductase test had excessively high counts
20 samples classified as fair by reductase test had excessively high counts
0 samples classified as poor by reductase test had low counts
0 samples classified as very poor by reductase test had low counts

2 samples classified as good by pH score had excessively high counts
3 samples classified as fair by pH score had excessively high counts
3 samples classified as poor by pH score had low counts
0 samples classified as very poor by pH score had low counts

SUMMARY

1. When plating methods are used, no one medium should be expected to give results which indicate correctly the condition of all grades of milk.

2. The pH score proved more efficient as a means of detecting slight changes in the history of milk than did the plating methods tried.

3. The methods as a whole were efficient in detecting slight changes in the history of the samples as follows:

In samples with bacterial counts under 25,000—45.8 per cent efficient.

In samples with bacterial counts between 25,000 and 100,000—71.4 per cent efficient.

In samples with bacterial counts between 100,000 and 1,000,000—85.0 per cent efficient.

In samples with bacterial counts over 1,000,000—97.0 per cent efficient.

It seems that the medium giving highest average counts depends upon the predominant groups present in the sample studied. This may account for divergent results obtained by various workers.

4. In 81 comparisons of the pH score with the reductase ratings and the bacterial plate counts, the pH score checked as well with the number of bacteria per cubic centimeter as did the reductase rating, although neither is an absolute measure of the bacterial content.

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INFLUENCE OF TWO PLANES OF FEEDING AND CARE UPON MILK PRODUCTION*

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When testing for the Advanced Registry and the Register of Merit was started at the dairy experiment station of the Bureau of Dairy Industry a number of years ago, it was noticed that the cows when on test produced a great deal more milk than they did when milked and fed only twice a day and otherwise managed in much the same way that any good dairyman might take care of his herd. This led to some specific experiments with certain animals to ascertain how much increase might be expected by keeping them under test conditions, and also to the tabulation of data regarding other animals which in the ordinary course of events were found to have made yearly records under each of the two conditions called for convenience "test" and "herd."

In brief, the test conditions were as follows: Keeping the cows in boxstalls, milking three times a day, feeding three times a day, feeding a large quantity of alfalfa hay and a small quantity of silage, feeding somewhat in excess of requirements as computed from the common feeding standards, feeding beet pulp, breeding to freshen about fifteen months from previous calving, and allowing no pasture. The herd conditions in the main were as follows: Keeping the cows in stanchions, milking twice a day, feeding twice a day, feeding smaller quantities of hay and a generous amount of silage, feeding only slightly more nutrients than called for by the standards, feeding no beet pulp, breeding to freshen about twelve months from previous calving, and allowing pasture to some of the cows.

Twenty-two cows included in this study have completed records under both conditions. One has made four records, six have made three records, while each of the remainder have made

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TABLE 1

Comparison of herd and test conditions

COW	BREED	AGE AT BEGINNING OF RECORD		HERD OR TEST CONDITIONS	LENGTH OF RECORD	DAYS PREGNANT	AVERAGE WEIGHT	GAIN IN WEIGHT	GRAIN	DRIED BEET PULP	HAY	SILAGE	PASTURE	ACTUAL PRODUCTION		PRODUCTION CORRECTED FOR AGE		INCREASE			
														Milk	Fat	Milk	Fat	Milk	Fat	Milk	Fat
		yrs	*mos.*		*days*		*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*days*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*per cent*	*per cent*
50	Gr. Hol.	4	2	Herd	365	105	1,067	4	3,207	0	1,977	10,625	69	9,323.7	345.93	11,099.6	411.82	3,821.7	156.01	33.5	36.5
		5	9*	Test	365	0	1,157	192	4,980	2,190	5,824	7,249	0	14,515.1	555.65	15,240.9	583.43				
		7	11	Herd	365	0	1,135	12	6,954.7	0	3,652	12,535.5	0	11,727.2	442.59	11,738.9	443.03				
51	Gr. Hol.	5	6	Test	320	11	1,163	109	4,201	1,716	5,176	6,227	0	16,562.2	633.80	17,555.9	671.83	5,604.0	226.08	46.9	50.7
		7	2	Herd	320	226	1,207	58	4,462.3	0	2,618	9,937.3	0	11,808.5	440.40	11,951.9	445.75				
64	Gr. Hol.	5	7	Test	365	190	1,213	134	5,317	2,182	5.716	6,121	0	15,012.8	572.87	15,913.6	607.24	6,191.3	224.26	63.7	58.6
		6	10	Herd	365	4	1,146	80	3,580.9	0	2.566.6	10,096.5	89	9,527.9	375.32	9,722.3	383.98				
82	Gr. Hol.	2	11	Test	365	144	986	159	3,463	2,162	4.944	5,706	0	10,534.0	388.45	14,789.7*	545.39*	4,061.1	119.01	32.3	24.1
		4	4	Herd	365	0	1,009	51	4,086	0	2,526	11,636	0	10,718.5	420.67	12,580.4	493.74				
		6	11	Test	365	204	1,226	134	5,788.5	3,277.5	5,064	6,009	0	18,130.7	666.78	18,493.3	680.12				
88	Gr. Hol	2	1	Herd	290	181	970	93	3,226	0	2,130	5,769	0	6,624.4	293.59	9,916.8	439.51	4,510.5	148.54	44.0	32.7
		3	2	Test	290	0	1,104	108	4,250	1,740	4,701	4,504	0	11,717.2	477.98	14,763.7	602.25				
		4	9	Herd	290	138	1,094	10	3,274	0	2,048	8,056.9	51	9,340.1	412.70	10,589.7	467.91				
91	Gr Hol	2	7	Test	365	100	1,172	63	4,857	2,172	5,304	5,576	0	15,565.8	608.66	21,013.8	821.69	11,893.1	459.47	130.4	126.8
		4	1	Herd	365	149	1,247	32	2,333.8	0	2,908	14.475	0	7,606.7	302.09	9,120.7	362.22				
94	Gr. Hol.	2	4	Test	365	140	1,044	304	5,029	2,087	5,506	4,632	0	14,984.7	474.02	20,978.6	663.63	5,462.6	187.88	35.2	39.4
		3	9	Herd	365	174	1,148	166	3,467.8	0	4,145	11,456	0	12,483.9	383.91	15,508.0	476.91				
		5	1	Test	365	215	1,310	139	5.484.5	3,096.5	5,228	5,268	0	19,231.7	610.96	20,962.6	665.95				
229	Hol.	2	7	Test	365	37			5,773	2,251	4,336	8,196	0	20.063.8	631.50	27,086.1	852.53	12,331.3	346.02	83.6	68.3
		6	1	Herd	365	68	1,212	38	4,949	0	3.827	13.771.5	0	14,105.6	484.22	14,754.8	506.51				
254	Hol.	3	7	Test	328	144	1,283	242	4,574	1,821	4,875	6,171	0	13,336.1	436.99	16,136.7	528.76	5,365.4	178.43	49.8	50.9
		5	0	Herd	328	174	1,306	190	3,683.7	294	2,228	8,325	107	9,694.2	315.30	10,771.3	350.33				
256	Hol.	2	6	Test	338	40	1,163	238	4,799.2	2,016.5	4,238	5,272	0	12,914.0	457.79	17,563.0	622.59	3,123.6	92.70	21.6	17.5
		4	1	Herd	338	164	1,255	107	4,125.9	100	2,598	6,116	123	12,042.5	441.93	14,439.4	529.89				
258	Hol.	2	5	Test	365	151	1.161	196	4.629.3	2,114	5,419	5,674	0	14,115.2	449.89	19,479.0	620.85	7,989.8	214.24	69.5	52.7
		3	9	Herd	365	210	1,256	241	3,420.9	0	2,720	6,448	147	9,248.8	327.32	11,489.2	406.61				
402	Jer.	2	3	Herd	365	186			2,581	0	2,068	8.327	69	6,325.6	361.10	7,790.2	444.70	5,297.1	292.25	68.0	65.7
		3	6	Test	365	119	857	213	4,313	2,143	4,398	6,336	0	11,185.7	646.45	13,087.3	736.95				

408	Jer.	6	5	Test	365	0	1,123	13	5,338 5	2,165 5	6,615	5,676	0	14,455 4	816 30	14 455 4	816 30	4,858.4	277 46	50 6	51.5
		8	8	Herd	365	0	1,080	53	4,862 3	0	1,832	7,568	153	9,539 4	535 61	9,597 0	538 84				
409	Jer.	6	1	Test	332	65	990	42	4,620	1,879	5,304	4,970	0	10,587 1	593 30	10,587 1	593 30	2,700 0	170 37	34.2	40 3
		7	7	Herd	332	231	1.033	25	3,396	0	1,464	6,871	165	7,863 4	421 66	7,887 1	422 93				
411	Jer.	2	6	Test	264	73	857	117	3,260	1,539	2,920	4,052	0	6,395 6	363 72	8,250 3	454 65	2,634 8	167.28	39.9	53 0
		3	9	Herd	264	168	843	59	2,992 8	0	1,935	6,718 5	0	6,386 3	313 18	7,127 6	349 53				
		4	10	Herd	264	117	852	20	2,018 5	0	2,567	6,308	0	5,767 5	267 15	6,090 3	282 10				
		6	0	Test	264	100	1.007	5	3,659	2,261 5	3,649	3,346	0	10,237 1	511 53	10,237 1	511 53				
426	Jer.	2	9	Test	334	241	763	142	3.082 5	1,570 5	3,888	5,032	0	7,256 8	472 58	9,071 0	576 55	2,315 9	140 41	32.8	31.4
		3	10	Herd	334	251	800	101	3,648 5	0	1,680 4	7,970	0	6,351 7	402 01	7,057 5	446 68				
		5	11	Test	334	0	915	86	4,269	2,430	4,395	4,262	0	9,486 2	591 71	9,675 9	597 63				
427	Jer.	2	3	Herd	365	126	640	99	2,586 2	0	2,632	9,274	0	5,252 4	265 36	6.468 5	326 80	5,636 9	308 83	87 1	94 5
		3	9	Test	365	154	862	98	4,774 8	1,649 5	3,906	5,545	0	10,618 8	572 64	12,105 4	635 63				
436	Jer	2	10	Test	365	255	985	199	3,922 4	1,729 5	4,588	5,493	0	8,498 2	461 15	10,537 8	557 99	3,986 0	176.98	51 8	41 4
		3	11	Herd	365	213	982	75	2,751	0	1,775 5	7,978	151	6,962 7	386 80	7,702 1	427 88				
		5	1	Test	365	146	998	136	4,853 5	2,873 5	4,357	5,232	0	12,227 0	632 76	12,838 4	651 74				
438	Jer.	2	8	Test	306	137	999	98	3,514	1,390 5	3,565	3,740	0	7,513 3	426 51	9,466 8	524 61	4,109 6	198 54	76 7	60 9
		3	11	Herd	306	174	1,053	28	2,634 1	0	1,826 5	1,448 5	115	4,842 9	294 77	5,357 2	326 07				
441	Jer.	2	4	Test	365	128	958	278	3,489 5	1,754 5	4,339	5,674	0	6,439 5	397 69	8,500 1	509 04	−72 1	37 77	−0 8	8 0
		3	9	Herd	365	37	1.058	134	4,313 8	0	2,738	9,649	0	7,680 7	422 26	8,572 2	471 27				
467	Jer.	5	7	Test	365	180	1,098	130	4,570 8	1,970	4,326	5,700	0	10,814 5	561 38	11,138 9	572 61	3,065 9	174 07	38 0	43 7
		6	10	Herd	365	103	1,004	53	3,773 4	0	2,333	9,388	53	8,000 3	394 95	8,073 0	398 54				
485	Jer.	2	2	Test	365	190	797	174	5.014 2	1,702	3,933	4,752	0	9,917 0	556 64	13,388 0	729 20	3,731 2	213 73	38 6	41 5
		3	4	Herd	365	100	847	64	3,961 6	0	2,186	3,623	173	8,449 7	451 04	9,656 8	515 47				
Average				Herd		134	1,053	83								Total average				51.2	49 5
				Test		109	1,064	141								Total average of all cows except 91 and 441				49.8	47 8

* Eight per cent added on account of being milked one-half the time twice per day.

two records. Of the 52 records, 27 were made under test conditions and 25 under herd conditions. When a cow had more than one record under the same conditions, the records were averaged before a comparison with the record under the other set of conditions was made. The production of milk and butterfat was corrected for age by applying Clark's (1) factors to both the Holsteins and Jerseys under herd conditions, Gowen's (2) factors to Holsteins under test conditions, and Graves and Fohrman's (3) figures to Jerseys under test conditions.

While all the test records were for 365 days some of the herd records, because of approaching parturition, were somewhat less than this. The length of period used in these calculations was the same for the two conditions and was determined by the time the cows continued to give milk under herd conditions. The average length of period, therefore, was 346.5 days instead of 365 days. Although it was realized that making calculations in this way worked to the disadvantage of the test cows, still it was thought that carrying the records for a full year with the herd cows dry part of the time would give undue weight to the influence of pregnancy. The average number of days from conception to the end of the records as shown in this report was 109 days for the test cows and 134 days for the herd cows. Since the difference in the length of time that the calves were carried was only 25 days, it is believed that the matter of pregnancy exerted but little influence on the results as given.

The weights of the cows in the regular herd were taken for three consecutive days on the first, second, and third days of each month; the weights of the test cows were taken on these same days and also on the fifteenth, sixteenth, and seventeenth of each month. The weights as reported are an average of such three days as were believed to represent most accurately the weights at the beginning and end of the test.

The grain mixture for the test cows was for the most part made up as follows: 200 pounds ground oats, 150 pounds linseed meal, 75 pounds cottonseed meal, 100 pounds gluten feed, 100 pounds, hominy feed, 100 pounds wheat bran, and salt at the rate of 1 per cent. The grain mixture for the herd contained less protein and no gluten feed. It was made up of 100 pounds ground oats, 50 pounds linseed meal, 50 pounds cottonseed meal, 100 pounds hominy feed, 100 pounds wheat bran, and salt at the rate of 1 per cent. The hay was at least average quality for Eastern alfalfa, and any small difference in quality was in favor of the test cows. The silage was made from corn and was uniformly good. The pasture was a mixture of grasses and clovers with orchard grass predominating. It varied in quality, being good early in the summer and fair later in the season.

The following facts are shown in table 1: The cows on test gained more in body weight than those under herd conditions, which was to be expected in view of the more liberal feeding of the test cows. The percentage of butter fat in the milk was unchanged. The average actual production for test cows was 12,446.6 pounds milk and 544.67 pounds butterfat, and for herd cows 8,776.8 pounds milk and 384.74 pounds butterfat. The average increase of test cows over herd cows, as shown by the corrected figures, is 51.2 per cent milk and 49.5 per cent butterfat. By leaving out one abnormally high cow (no. 91) and one abnormally low cow (no. 441) the averages are 49.8 per cent milk and 47.8 per cent butterfat.

As a rule the greater the production the greater the profit, but this applies to animals kept under similar conditions and does not necessarily hold in the present study. It is a matter of prime importance for the dairyman to know the conditions under which his cows yield him the greatest profit. The following discussion has this as its object.

The average prices paid at this stations for feed and bedding for the past five years are as follows:

Wheat bran	$32.79 per ton
Linseed meal	52.49 per ton
Cottonseed meal	51.74 per ton
Hominy feed	36.50 per ton
Ground oats	42.71 per ton
Dried beet pulp	41.43 per ton
Gluten feed	47.04 per ton
Alfalfa hay	29.90 per ton
Wheat straw	14.04 per ton
Corn silage	8.00 per ton
Pasture per cow	0.12 per day

The labor has been estimated at 40 cents a day for the test cows by assuming that a man who is paid $4.00 for a ten-hour day will milk and care for 10 test cows. It has also been estimated from the work at the station and from correspondence with dairymen that the cost of milking and caring for a cow in the regular herd is about one-half that required for a test cow. The bedding required for a cow in a boxstall is about 13 pounds of straw every day; while only 4 pounds is needed for a cow confined in a stanchion, and that amount is needed only during the winter. The value of the milk has been estimated at 30 cents and 40 cents a gallon, and the profits are figured on the basis of both prices.

From tables 1 and 2 it will be noted that under the conditions which prevailed at this station when the value of the milk is figured at 40 cents a gallon it makes little difference in the total profits whether cows are kept under test or herd conditions. However, if the milk is figured at 30 cents a gallon the cows under herd conditions show considerable greater profit. The higher the price of milk and the lower the cost of feed and labor, the more favorable the test conditions appear. At the prices which prevail here for feed and labor, milk selling for more than 40 cents a gallon will show more profit for test conditions, while milk at less than 40 cents will show more profit for herd conditions.

TABLE 2

Comparison of profits from 22 cows kept under test conditions and from the same cows under herd conditions when milk sells at 40 cents and 30 cents a gallon

	COST OF FEED	COST OF LABOR	COST OF BEDDING	TOTAL COST OF PRODUCTION	VALUE OF MILK AT 40 CENTS A GALLON	PROFIT AT 40 CENTS A GALLON	VALUE OF MILK AT 30 CENTS A GALLON	PROFIT AT 30 CENTS A GALLON
Test	$5,108.39	$3,048.80	$695.59	$8,852.78	$12,732.84	$3,880.06	$9,556.47	$703.69
Herd	3,373.10	1,524.40	98 78	4,996.28	8,978.68	3,982 40	6,738 84	1,742.56

Possibly a better way to estimate the profit from cows kept under test conditions as compared with those under herd con-

TABLE 3

Increase (+) or decrease (−) in feed and labor cost of keeping cows under test conditions, as compared with herd conditions, at each of four price levels

UNIT ON WHICH PRICE IS BASED	QUANTITIES		PRICES PER UNIT AND INCREASE IN COST FOR QUANTITIES USED							
			(1)		(2)		(3)		(4)	
			Price	Increased cost	Price	Increased cost	Price	Increased cost	Price	Increased cost
Ton	Pounds of grain	64,940	$25	$811.75	$35	$1,136 45	$45	$1,461.15	$55	$1,785 85
Ton	Pounds of hay	50,419	10	252 09	20	504 19	30	756 28	40	1,008 38
Ton	Pounds of silage	−67,464	4	−134.93	6	−202.39	8	−269.86	10	−337.32
Day	Days on pasture	−1,404	0 06	−84 24	0 12	−168.48	0 18	−252 72	0 24	−336.96
Ton	Pounds of straw	76,220	5	190.55	10	381 10	15	571.65	20	762.20
Hour	Hours of labor	3,811	0 20	762.20	0 30	1,143 30	0 40	1,524 40	0.50	1,905 50
Total				$1,797.42		$2,794.17		$3,790 90		$4,787.65
Cost of extra milk per 100 pounds				$2.23		$3 46		$4.70		$5.93
Cost of extra milk per gallon				0 19		0 30		0.40		0.51
Cost of extra butterfat per pound				0.51		0 79		1.08		1.36

ditions is to find the amount of extra feed and labor required, and then from the difference in production find the cost of the extra production per gallon of milk or pound of butterfat. Table 3 shows four sets of costs varying from those which might obtain in some sections where the farmer raises practically all his feed for cows to those where the dairyman buys all his feed except pasture. By using that set of conditions which most nearly applies to his own case, the dairyman can estimate fairly well whether it will pay him to give his animals test-cow care.

DISCUSSION OF RESULTS

This investigation shows that cows kept under the test conditions which prevail at the Beltsville Station yield approximately 50 per cent more milk and butterfat than cows kept under herd conditions. This is an important point to remember in buying stock on the basis of records. A 400-pound record under herd conditions is equal to 600 pounds under test conditions.

The quantity of production, the cost of feeding and caring for the cows, and the value of the product determine whether or not it will pay to give cows the same care as if on test. With cows such as were used in this work it is obvious that test-cow care and feeding will not pay if the product is to be disposed of for butter making even if both feed and labor are cheap. Since the labor cost per unit of product is greater with lower producing cows, it is evident that the poorer the cows the less likely is the test-cow care to be profitable. As the price of the product increases the stronger becomes the likelihood of test conditions being profitable. Doubtless some dairies which receive high prices for milk could increase their profits by giving their herds test-cow care and feed. In general, however, the herd method of caring for cows is the more profitable.

A great deal has been written about the cost of running a cow on yearly test. Under the conditions of this experiment it is shown that if a dairyman receives 40 cents a gallon for 4.4 per cent milk at the barn and the average yield is around 550 pounds of butterfat a year, about the only cost of testing is that of the supervisor. If a dairyman receives 30 cents a gallon the cost of testing each cow will be about $50 in addition to the cost of the supervisor.

REFERENCES

(1) Clark: Jour. Dairy Sci., vii, 2, p. 547.
(2) Gowen: Milk Secretion, Table 17, p. 55.
(3) Graves and Fohrman: U. S. Dept. of Agr. Bul. 1352.

A GRAPHICAL METHOD OF PROPORTIONING AND STANDARDIZING ICE CREAM MIXES*

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This graphical method of proportioning an ice cream mix is believed to be simple, rapid, and of special value, because it illustrates the principle underlying all methods of standardizing.

To illustrate the use of the method it will be assumed that a desired mix is to have 10 per cent fat, 11 per cent msnf, 14.5 per cent sugar, and 0.5 per cent gelatine, and that it must weight 3000 pounds.

The available milk products have the following compositions:

MILK PRODUCT	FAT	MSNF
	per cent	*per cent*
Cream	30.0	6.3
Milk	4.0	8 8
Condensed milk	1.0	27.0

The number of pounds of sugar and gelatine are calculated as usual:

3000 pounds of mix × 14.5 per cent sugar = 435.0 pounds of sugar
3000 pounds of mix × 0.5 per cent gelatine = 15.0 pounds of of gelatine
Total = 450 pounds of products not milk

The pounds of fat and milk solids not fat are determined in the same manner as the sugar and gelatine:

3000 pounds of mix × 10.0 per cent fat = 300 pounds of fat
3000 pounds of mix × 11.0 per cent msnf = 330 pounds of milk solids not fat

The pounds of milk products in the mix are determined by deducting the pounds of products not milk from the total amount of mix.

3000 pounds of mix − 450 pounds of sugar and gelatine = 2550 pounds of milk products

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In the 2550 pounds of milk products, which will be made up of cream, milk, and condensed milk, there will be 300 pounds of fat or

$$\frac{300}{2550} \times 100 = 11.764 \text{ per cent fat}$$

and 330 pounds of msnf or

$$\frac{300}{2550} \times 100 = 12.941 \text{ per cent milk solids not fat}$$

The graph used with this method of proportioning the mix is illustrated in figure 1. The per cent of fat is plotted on the vertical axis and milk solids not fat on the horizontal axis. The composition of the cream is shown on the graph by the point *A*. The compositions of the milk and condensed milk are represented by the points *B* and *C* respectively. The per cent of fat and milk solids not fat desired in the 2550 pounds of milk products is indicated by the point *D*. Points *A* and *B*, when connected with a straight line, form the line *AB*, which represents the compositions of all the possible mixtures which can be made by combining cream and milk of the compositions *A* and *B*. A straight line drawn through the points *C* and *D* and extended intersects the line *AB* at *E*. The point *E*, according to the estimate made from its location on the graph, indicates the composition of a mixture of milk and cream with 15.82 per cent fat and 7.66 per cent msnf. The line *CE* represents the compositions of every possible combination of the 15.82 per cent milk and cream mixture and the 1.0 per cent condensed milk. Since the point *D* is obviously the composition of one of these possible mixtures, and since *D* also represents the composition desired in the 2550 pounds of milk products, it follows that the mix can be made by combining the 15.82 per cent mixture of the milk and cream with the 1.0 per cent condensed milk.

The next step in the procedure is to determine the pounds of the milk and cream mixture and condensed milk which combined will make 2550 pounds of mix containing 11.764 per cent fat. The pounds of milk and cream necessary to make the amount of 15.82 per cent mixture required for combination with the condensed milk can then be calculated.

No attention to the milk solids not fat content of the milk products is necessary in the calculations *after* the compositions

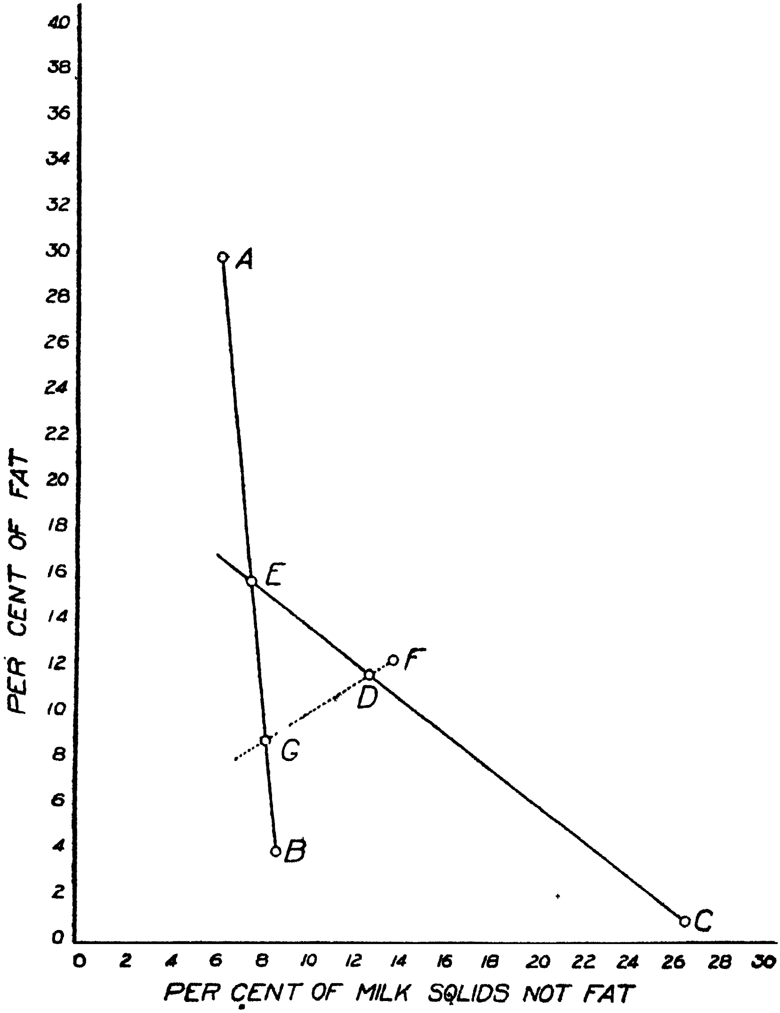


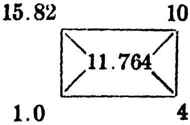
FIG. 1. GRAPH FOR PROPORTIONING AND STANDARDIZING ICE CREAM MIX

This graph was drawn on Keuffel and Esser standard cross section millimeter paper using the scale: 10 millimeters = 1.0 per cent.

of the milk products have been correctly plotted on the graph. This simplifies the problem to a great extent because any method for standardizing for fat alone can be used to proportion the ingredients of the mix.

The steps in the calculations by the Pearson Square Method are as follows:

1. Calculate the pounds of 15.82 per cent milk and cream mixture and 1.0 per cent condensed milk necessary to make 2550 pounds of 11.764 per cent mix.



10.764 unit parts of 15.82 per cent milk-cream mixture

4.056 unit parts of 1.0 per cent condensed milk

14.820 unit parts of 11.764 per cent mix

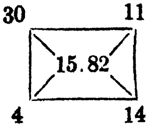
$$\frac{\text{Pounds of condensed milk}}{2550} = \frac{4.056}{14.820}$$

$$\text{Pounds of condensed milk} = \frac{4.056}{14.820} \times 2550 = 697.89 \text{ pounds}$$

The pounds of condensed milk deducted from the total number of pounds of milk products leaves the pounds of milk and cream necessary in the mix.

2550 pounds of milk products − 697.89 pounds condensed milk = 1852.11 pounds of milk and cream testing 15.82 per cent fat

2. Calculate the pounds of 30 per cent cream and 4 per cent milk necessary to make 1852.11 pounds of milk-cream mixture testing 15.82 per cent fat.



11.82 unit parts of 30 per cent cream

14.18 unit parts of 4 per cent milk

26.00 unit parts of 15.82 per cent milk-cream mixture

$$\frac{\text{Pounds of cream}}{1852.11} = \frac{11.82}{26.00}$$

$$\text{Pounds of cream} = \frac{11.82 \times 1852.11}{26.00} = 842.01 \text{ pounds}$$

The pounds of cream deducted from the pounds of milk-cream mixture required in the mix leaves the pounds of milk necessary.

1852.11 pounds of milk and cream−842.01 pounds of cream=1010.10 pounds of 4 per cent milk

Proof of calculations in pounds

	MATERIALS	FAT	MSNF	SUGAR	GELATINE
Condensed	697.89	6.979	188.433		
Milk	1010.10	40.404	53.046		
Cream	842.01	252.600	88.889		
Sugar	435.00			435	
Gelatine	15.00				15
Total	3000.00	299.983	330.368	435	15
Per cent	100.00	9.999	11.012	14.5	0.5

It may happen that a mix which contains wrong amounts of fat and milk solids not fat must be corrected by proper combination with other milk products.

Assume that the 3000 pounds of mix whose formula has already been proportioned contains 10.54 per cent fat and 11.90 per cent msnf, 14.5 per cent sugar, and 0.5 per cent gelatine, and that the milk products available for adjusting the mix to the desired composition of 10.0 per cent fat, 11.0 per cent msnf, 14.5 per cent sugar, and 0.5 per cent gelatine are the same as those used to make the original mix.

It is necessary to know first the per cent of fat and milk solids not fat in the milk products portion of the 3000 pounds of incorrect mix.

3000 pounds mix × 10.54 per cent fat = 316.2 pounds of fat
3000 pounds mix × 11.90 per cent msnf = 357.0 pounds of milk solids not fat

It has been shown that this 3000 pounds of mix contains 14.5 per cent sugar and 0.5 per cent gelatine as the only ingredients not milk products and that there are, therefore, 2550 pounds of milk products in the mix. Since the milk products portion of the mix is the only source of milk fat and milk solids not fat, then in this 2550 pounds portion of the theoretically sugar and gelatine free mix the per cent of fat will be:

$$\frac{316.2}{2550} \times 100 = 12.4 \text{ per cent fat}$$

and the per cent of milk solids not fat will equal:

$$\frac{357.0}{2550} \times 100 = 14.0 \text{ per cent msnf}$$

The calculations for the original correct mix show that the 2550 pounds of milk products should contain 11.764 per cent fat and 12.941 per cent msnf. It is necessary, therefore, to add cream and milk to the incorrect mix. The reason for this particular selection of materials will be mentioned later. If the composition of the milk products portion of the incorrect mix is plotted on the graph, it will fall at point *F*. If the line determined by the points *F* and *D* is drawn *from F through D*, it will intersect the line *AB* at *G*. The point *G* represents on the graph the composition of a mixture of 30 per cent cream and 4 per cent milk containing 8.89 per cent fat and 8.30 per cent msnf.

The procedure explained in connection with the proportioning of the first mix is followed again in this standardization.

1. Calculate the pounds of 8.89 per cent milk-cream mixture necessary to add to 2550 pounds of 12.4 per cent mix to give a mixture testing 11.764 per cent.

12 40 2 874 unit parts of milk-cream mixture
11.764
8.89 0 636 unit parts of milk products portion of incorrect mix

The total number of pounds of milk-cream mixture necessary to standardize the 2550 pounds of the theoretically sugar and gelatine free mix will be:

$$\frac{2550}{2.874} \times 0\ 636 = 564\ 3 \text{ pounds of milk-cream mixture testing 8.89 per cent fat}$$

2. Combine the 30 per cent cream and the 4 per cent milk to make 564.3 pounds of a mixture testing 8.89 per cent fat:

30.0 4 89 unit parts of cream
8.89
4.0 21.11 unit parts of milk

26.00 unit parts of milk-cream mixture

There are 564.3 pounds of milk-cream mixture required, therefore:

$$\frac{564.3}{26.00} \times 4.89 = 106.13 \text{ pounds of cream}$$

and

$$\frac{564.3}{26.00} \times 21.11 = 458.17 \text{ pounds of milk}$$

The summary of the calculations in pounds

	MATERIALS	FAT	MSNF
Incorrect mix	3000.00	316.20	357.00
Cream	106.13	31.83	6.68
Milk	458.17	18.33	40.32
Total	3564.30	366.36	404.00

Only 10 per cent of fat is desired in the corrected mix which, if it contains 366.36 pounds of fat, must weigh:

$$\frac{366.36}{10.0} \times 100 = 3663.6 \text{ pounds of corrected mix}$$

There are 3000 pounds of this mix which contains the desired amount of sugar and gelatine, leaving an excess of 663.6 pounds of the new mix still requiring sugar and gelatine. The amounts of each are calculated, as usual:

663.6 pounds excess mix × 14.5 per cent sugar = 96.22 pounds of sugar
663.6 pounds excess mix × 0.5 per cent gelatine = 3.32 pounds of gelatine

The proof of the calculations in pounds

	MATERIALS	FAT	MSNF	SUGAR	GELATINE
Incorrect mix	3000.00	316.20	357.00	435.00	15.00
Cream	106.13	31.83	6.68		
Milk	458.17	18.33	40.32		
Sugar	96.22			96.22	
Gelatine	3.32				3.32
Total	3663.84	366.36	404.0	531.22	18.32
Per cent	100.00	10.00	11.02	14.50	0.5

The form of graph illustrated in this explanation has one or two interesting characteristics and applications. The line *AE* and *EB* might be measured in units of length and the amounts of milk and cream calculated by the proportions:

$$\frac{\text{Pounds of milk}}{\text{Pounds of mixture}} = \frac{\text{Length of } AE}{\text{Length of } AB}$$

In the same way the amounts of condensed or milk-cream mixture can be calculated by measuring the proper lines on the graph:

$$\frac{\text{Pounds of condensed milk}}{\text{Pounds of milk products}} = \frac{\text{Length of } ED}{\text{Length of } EC}$$

In selecting the milk products to be used to standardize an off batch of mix, the procedure is not involved. The straight line drawn *from* the point representing the composition of the milk products portion of the incorrect mix *through* the point of composition of the desired mix will intersect the line drawn between the two points representing the compositions of the two milk products which alone can be used to balance the incorrect mix. When the addition of water alone is necessary to adjust the mix, this same straight line, if extended, will pass through the origin of the graph, that is, 0.0 per cent fat and 0.0 per cent milk solids not fat.

In practice, cross section paper mounted on a large drawing board can be used to good advantage; since a large graph is more accurate than a smaller one, when properly ruled. The points representing the compositions of the milk products can be fixed by inserting glass headed pins into the graph at the proper points. The lines can be quickly and accurately shown by stretching fine black thread between the pins. This procedure does not interfere with the accuracy of the method and makes it possible to use the graph for a long time without changing the paper.

ON THE CALCULATION OF THE FREEZING POINT OF ICE-CREAM MIXES AND OF THE QUANTITIES OF ICE SEPARATED DURING THE FREEZING PROCESS*

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In studying the physics of ice-cream freezing and in calculating refrigeration constants for ice-cream work, it is desirable to be able not only to calculate the freezing points of ice-cream mixes but also to determine the quantity of ice that may be separated from the mix at any temperature. Making certain allowable assumptions, it is possible to calculate these values with considerable accuracy.

Van Slyke (1) gives the composition of the average milk as follows:

		per cent
Fat		3.9
Milk solids		8.99
Lactose	4.9	
Protein	3.2	
Salts	0.89	
Water		87.11

Because milk is an animal secretion it is of necessity isotonic with the blood of the animal from which it comes. This means that the freezing point of milk is a fairly constant value, which for cow's milk may be taken as −0.55°C., or in other words, the freezing point of milk is 0.55°C. lower than that of water.

By employing the usual formula for obtaining the molecular weight of an un-ionized substance from the depression of the freezing point of a solvent it can be calculated that the milk sugar accounts for 0.306°C. of the normal freezing-point lowering of milk and that 0.244°C. is caused by the combined action of the milk salts, protein, fat, etc. Not taking into consideration the

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very small effect of the fat and protein upon the freezing point of milk, it is found from these data that the *apparent* molecular weight of the milk salts is 78.6, a figure which will be of considerable value in the calculations given later in this paper.

The molecular weight formula is as follows:

$$\Delta = K \frac{G}{M}$$

In this formula Δ represents the freezing point depression in degrees Centigrade; K the constant, depending on the molecular weight of the solvent (for water 18.6); G the weight of dissolved substance in 100 grams of water; and M its molecular weight.

From the analysis of milk previously given, it is seen that there are 4.9 parts of lactose to 87.11 parts of water, which is equivalent to 5.63 parts of lactose to 100 parts of water. The substitution of the values in the molecular weight formula gives the following:

$$\Delta = 18.6 \frac{5\ 63^{*}}{342.2}$$

$$\Delta = 0.306$$

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* For the purposes of this paper Van Slyke is considered to have expressed the lactose content of milk in terms of anhydrous lactose rather than in those of the monohydrate.

Subtracting 0.306 from 0.55 gives 0.244, the depression of the freezing point of water due to the salts of milk. By again using the molecular weight formula, the apparent molecular weight of the salts can be calculated by substituting 0.244 for Δ and, since there are 0.899 part salts to 87.101 parts water, or 1.032 parts salts to 100 parts water, by substituting 1.032 for G:

$$M = K \frac{G}{\Delta}$$

$$M = 18.6 \frac{1.032}{0.244}$$

$$M = 78.6$$

From the molecular weight formula and from a knowledge of the composition of an ice-cream mix, the depression of the freezing point of the mix that is caused by the salts can be calculated.

TABLE 1

Freezing point lowering of cane sugar solutions

PARTS CANE SUGAR TO 100 PARTS WATER	PER CENT CANE SUGAR	LOWERING	LOWERING DUE TO 1 PART CANE SUGAR
		°*C.*	
3.59	3.47	0.21	0.05
6.85	6.41	0.40	0 05
10.84	9.78	0.65	0.06
15 83	13 67	0 95	0.06
19.80	16.53	1.23	0.06
22.58	18.42	1.37	0.06
25.64	20.41	1.58	0.06
28.51	22.19	1.77	0.06
32.22	24.37	1.99	0.06
35.14	26.00	2.15	0.06
37.86	27.46	2.33	0.06
43.72	30.42	2.71	0.06
45.62	31.33	2.82	0.07
50.02	33.35	3.13	0 07
54.74	35.37	3.47	0.07
59.46	37.29	3.81	0 07
64.55	39.23	4.22	0.07
69.74	41.09	4.60	0 07
75.91	43.15	5.07	0 07
82.35	45.16	5.65	0.07
88.67	47.00	6.11	0.07
95.94	48.97	6.76	0.07
102.70	50.65	7.38	0.07
111.30	52.67	8.06	0.07
121.00	54.75	9.02	0.07
131.60	56.82	9.93	0.07
143.10	58.86	10.90	0.07
153.80	60.60	11.69	0.08
165.60	62.35	12.72	0.08
181.70	64.49	13.80	0.08

The other ingredients of the mix that will affect the freezing point are the two sugars, sucrose and lactose. By using the method previously given, it would be possible to calculate the freezing-point depression of an ice-cream mix caused by these sugars if it had not been shown (2) that sucrose in concentration does not obey the freezing-point law. To find the freezing-point depression then, it is necessary to calculate the *total* sugar concentration of an ice-cream mix, on the water basis, and refer to the actual freezing-point curve for cane-sugar solutions, which has been worked out quite carefully by Pickering (3) and checked by P. N. Peter (4) of these laboratories. By adding to this freezing-point depression the depression caused by the salts, a very close approximation of the true freezing point of the mix is obtained.

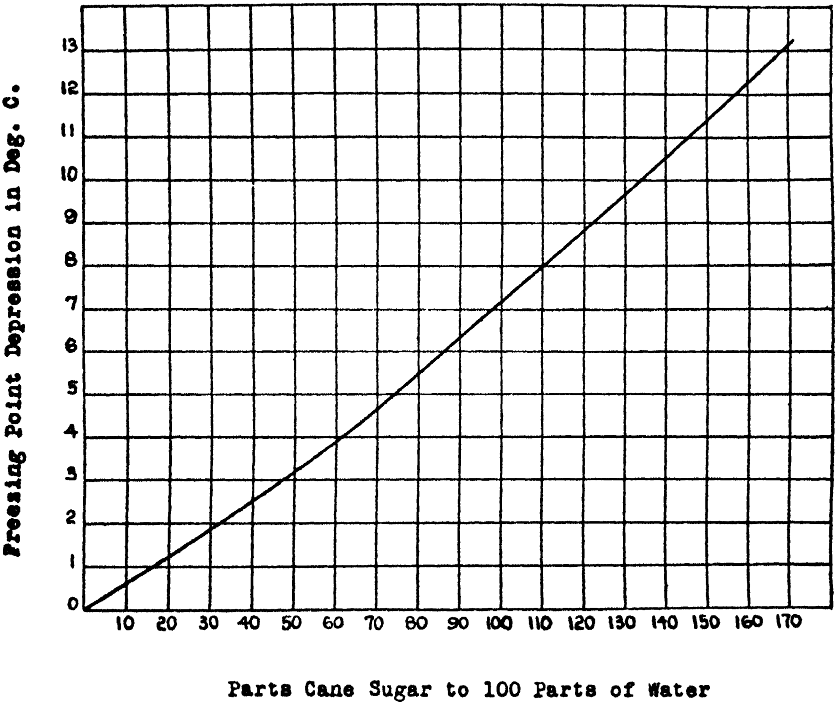

FIG. 1. FREEZING-POINT DEPRESSIONS OF CANE SUGAR SOLUTIONS

Two assumptions have been made here which have not yet been proved experimentally: First, that the freezing-point depression caused by the salts obeys the freezing-point law, which is probably true because of the moderately concentrated solutions encountered; second, that mixtures of lactose and cane sugar, where lactose is present in moderate amounts, will closely follow the freezing-point curve of pure cane sugar in water solution, because the molecular weight of the lactose (anhydrous) is the same as that of cane sugar.

The simplest way of testing the validity of these assumptions is to calculate and determine experimentally the freezing points of some actual ice-cream mixes. For this calculation the preceding method can be simplified somewhat.

Multiplying the number parts milk solids not fat in the mix by 0.545 gives the lactose content. Adding to this the cane-sugar content, multiplying by 100 and dividing by the number parts water give the number parts sugar per 100 parts water. This may be expressed as follows:

$$\frac{(\text{Milk solids not fat} \times 0.545 + \text{Sucrose})\,100}{\text{Water}} = \text{Parts sugar to 100 of water}$$

Referring then to the freezing-point curve of sugar, the corresponding freezing-point depression (A) is found.

Dividing the number parts milk solids by ten gives the salts content of the mix. Multiplying by 100 and dividing by the water content give the number parts salts to 100 parts water, and this multiplied by 18.6 and divided by 78.6 give the freezing-point depression of the salts. This may also be expressed as follows:

$$\frac{\text{M.S.N.F.} \times 100 \times 18.6}{10 \times \text{Water} \times 78.6} = \text{Freezing point depression due to salts } (B)$$

or

$$\frac{\text{M.S.N.F.} \times 2.37}{\text{Water}} = B$$

$$A + B = \text{Total Lowering}$$

These formulas are now applied to the calculation of the freezing points of several ice-cream mixes made up in such a way that the only difference between them is in the water concentration. This is done in order to calculate and check the freezing-point curve of a certain basic mix which had the following concentration:

		parts
Total solids		36.03
Fat	12.5	
Sugar (sucrose)	14.0	
Milk solids not fat	9.53	
Water		63.97

By concentration in the vacuum pan, samples of this mix were made which had one-eighth, one-quarter, three-eighths, and one-half of the water removed. The freezing points of these concentrations were determined with the Beckman thermometer in a standard freezing-point apparatus and define the freezing point curve of the basic mix. Table 2 gives the results of these measurements as well as of the calculations made by the formulas previously outlined.

TABLE 2

Results of calculations and measurements of freezing points of experimental ice-cream mixes

NUMBER	FAT	M.S.N F	CANE SUGAR	WATER	TOTAL SUGAR TO 100 H_2O	F P DEP DUE TO SUGAR *A*	F P DEP DUE TO SALTS *B*	CAL F P. $(A + B)$	F P
	parts	*parts*	*parts*	*parts*	*parts*				
1	12.5	9.53	14.00	63.97	30.00	1.90	0.35	−2.25	−2.29
2	12.5	9.53	14.00	55.98	34.29	2.12	0.40	−2.52	−2.54
3	12.5	9.53	14.00	47.98	40.00	2.47	0.47	−2.94	−3.02
4	12.5	9.53	14.00	40.00	47.86	2.98	0.56	−3.54	−3.62
5	12.5	9.53	14.00	31.99	60.00	3.83	0.71	−4.54	−4.49

It will be seen that there is a very fair agreement between the calculated values and those obtained experimentally.

Experiments appear to justify fully the assumption that the temperature of freezing of an ice-cream mix concentrated in the vacuum pan is the same as the equilibrium temperature that must be attained when the unfrozen portion of the partially frozen unconcentrated mix has the same concentration as the vacuum-prepared sample. These experiments seem to show that ice-cream mixes can be moderately concentrated in the pan

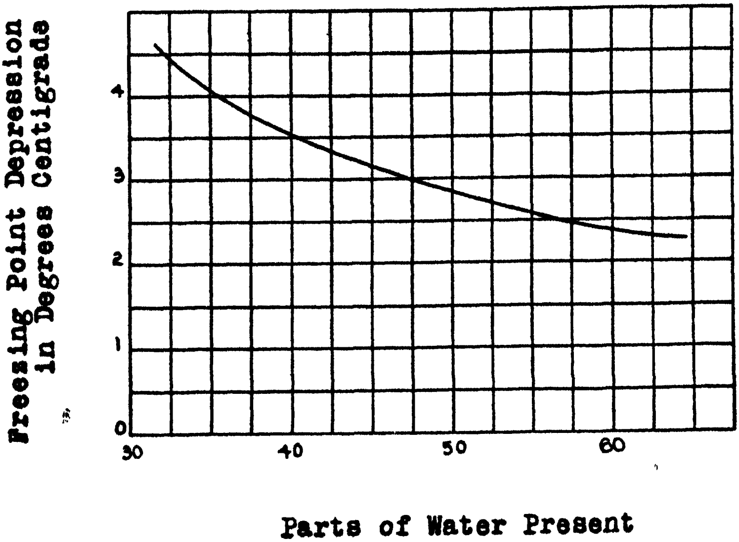

FIG. 2. FREEZING-POINT DEPRESSIONS OF MIXES PLOTTED AGAINST WATER CONCENTRATION

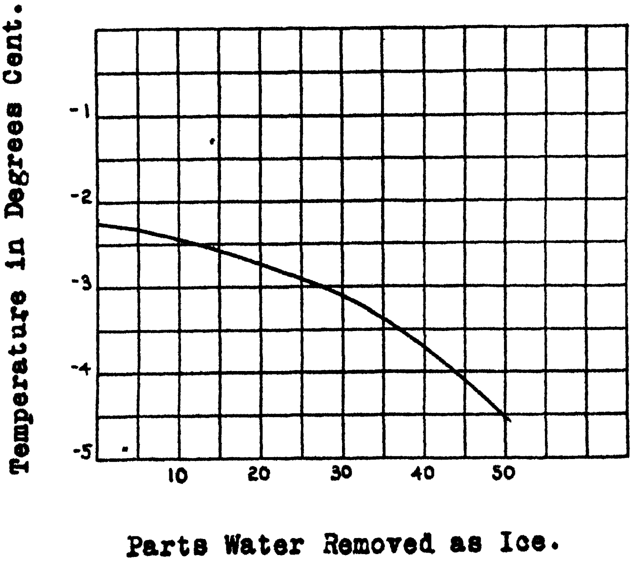

FIG. 3. QUANTITIES OF ICE SEPARATED FROM EXPERIMENTAL MIX AT DIFFERENT TEMPERATURES

and rediluted to normal concentration without any apparent effect upon the physical properties of the mix.

In figure 2 is plotted the freezing point depressions of the experimental mixes against the water concentrations. In figure 3 the freezing points are plotted against the portions of water removed from the basic mix. This latter curve can be used to determine the quantity of ice that may be separated from the ice-cream mix at any freezing temperature. The best way therefore, to determine the quantity of ice that may be separated from any mix at any temperature is to calculate the freezing points of the mix at a number of concentrations, then to plot the freezing-point water-removed curve and from this to estimate the quantity of ice that is frozen out at any one temperature.

Two questions would now naturally arise. First, with the supercooling of the mix in the freezer, how can the equilibrium temperature be determined? Second, may milk sugar crystallizing in the freezer invalidate our calculations, since these calculations are really based upon the assumption that ice is the only material to be thrown out in solid form during the period that the mix is in the freezer?

Unpublished experiments carried out by O. E. Williams and the author show that if a small portion of ice cream is removed from the freezer and its temperature determined, this sample is no longer supercooled and the temperature is very close to the equilibrium temperature. This is true because the quantity of ice separating to attain equilibrium after the portion is removed from the freezer is negligible.

In answer to the second question it can be said that the tendency of lactose to crystallize in the freezer will probably be very slight. This conclusion is reached from the following consideration. Hudson (5) has shown that when lactose is in equilibrium in solution, one part is in the hydrated form and one and one-half parts in the anhydrous form. The anhydrous form is very soluble. When lactose crystallizes, the hydrate deposits, some of the anhydride turns to hydrate and also deposits, the rate of separation then being governed by the rate of transformation, which at low temperatures is shown to be very slow.

The most concentrated mix considered here has about sixteen parts of lactose to 100 parts of water. At −4.5°C. probably about nine parts are soluble. This would mean that our solution is supersaturated to at least seven parts. However, since at least four of these are in the anhydrous form, this solution, while it is potentially supersaturated to at least seven parts, is actually supersaturated to but three. In view of the great tendency (6) of lactose to supersaturate, it seems unlikely that any should crystallize in the freezer. Even if this should happen, calculation shows that the resultant error in determining the quantity of ice that would be frozen at this temperature (−4.5°C.) is about one per cent. At higher temperatures this error would be proportionately less.

In giving the method for calculating the freezing points of ice-cream mixes and in showing how the quantity of ice that may be separated from a mix at any temperature in the freezer can be determined, no reference has been made to the effect of flavoring material on the freezing point of the basic mixes. However, if the water concentration, freezing point, and quantity of flavoring material added to the mix are known, the effect on the freezing points of the basic mixes may be calculated by the principles outlined in this paper. Allowance must be made if any milk product used in making the mix is low in lactose content.

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SOFT CHEESE INVESTIGATIONS*

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INTRODUCTION

The literature contains only few and incomplete references to the manufacture of cream cheese. This is a definite variety of cheese; the process having been evolved from the modification of other soft cheese methods. In consequence, it is possible at present to find this cheese made by different methods; and the result is that our commercial cream cheese varies in quality.

Regardless of the method employed, the cream cheese manufacturers desire a smooth texture and mild flavored cheese. The processes employed at the present time have been perfected with these two objectives in view.

SCOPE OF PRESENT INVESTIGATION

The studies recorded were conducted to observe the methods and result of certain phases of soft cheese making. For these observations it was necessary to prepare curds by numerous methods. The studies relating to soft cheese in general were conducted with curds prepared by a Neufchatel process or this process slightly modified.

The development of a smooth texture and a mild flavor in cream cheese were the objectives of paramount value in this work. It was also essential in the cream cheese studies to develop a cheese which could be sliced. These points were investigated with curds prepared by a cream cheese process or this process slightly modified. Process modifications were employed only in order to obtain added knowledge of methods investigated. For this reason some of the analyses made for comparative purposes do not conform to the normal composition of soft cheese varieties.

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In the recording of data the composition of the milk or cream and the composition of the resulting cheese and whey were recorded for each series under observation. In each series a complete record of the work was recorded.

Milk and cream varying widely in fat content were used during the investigation. Process modification could be observed to better advantage by allowing a wide range of fat content of the milk and cream. For example, setting temperature variation influences could be observed more closely in milk than in cream.

The milk and cream used were analyzed for total solids. Fat determinations were made by the Röse-Gotlieb method. A record was also made of the age, temperature, flavor, and source of the milk. For each lot of cheese 35 or 50 pounds of milk or cream were used.

Curds were analyzed for total solids and fat. Curd yields and quality were recorded. Whey analyses for total solids and fat were made. Due to unavoidable mechanical losses the pounds of whey plus curd did not equal the pounds of milk or cream used. To avoid further mechanical losses the curds were not subjected to pressure.

Homogenization pressures as affecting the resulting curd were studied. The size of fat globules and their influence upon the cheese process were included with the homogenization studies. This also included observations on the clumping properties of the fat globules.

Series of skimmed milk were homogenized in order to determine whether or not homogenization would produce a smooth curd from milk in the absence of fat.

Rennet variations, the use of commercial cutures, and the temperature limits for setting the milk and cream were studied.

Textures, flavors, and keeping quality of the cheese as related to the composition of the milk or cream were investigated.

Pasteurization variations and their influence upon the resulting cheese and whey were investigated. Low and high pasteurization temperatures were employed with low and high fat content milk and cream. The results of the variations upon the cheese texture were very significant.

Several commercial brands of cream cheese were analyzed during the investigation. A report of their composition, texture, flavor and keeping quality is recorded.

A cream cheese process was perfected from the studies conducted at the Station. This method was compared with two methods used by the industry at the present time. An attempt was made to perfect the method commonly employed in draining the curds in cheese-cloth bags.

SOURCES OF MILK AND CREAM

All the milk used in the work was obtained from the Station herd. The cream was also obtained from the Station milk supply. The Station milk used averaged over 5 per cent milk fat, and more than 15 per cent of total solids. This explains the large yields obtained under certain trial conditions as the curds were not subjected to pressure as previously stated.

EQUIPMENT USED

The heat treatment of milk was done in a Creamery Package Horizontal coil pasteurizer. A Burrell surface cooler was also used during the heating and cooling process.

A small Gaulin homogenizer was used for all the homogenization work.

Standard shot gun cans were employed in setting the milk. A simple drain rack was designed to hold the curd from three lots of milk.

The drain sacks were made of cheese-cloth. A close study revealed that this cloth was made of threads 0.28 mm. wide with spaces between them 0.34 mm. square.

The analytical work was performed with standard equipment and according to methods described in Standard Methods of Milk Analysis or in the Official and Tentative Methods of Analysis of the Association of Agricultural Chemists.

EXPERIMENTAL

Effects of homogenization

Five series were prepared for the expressed purpose of studying the homogenization of milk and its effect upon the resulting products. In each series a normal milk was compared with milks homogenized at 2500 and 4500 pounds pressure. For all comparisons reported in this work, unless otherwise stated, the milk was pasteurized at 145°F. (62.8°C.) and held for thirty minutes.

TABLE 1

Showing averages of five series of cheese prepared to compare influences of homogenization

FACTORS STUDIED	HOMOGENIZATION PRESSURE		
	None	2500 pounds	4500 pounds
Per cent milk fat in milk	6 06	6 06	6.06
Per cent solids in milk	15.88	15.88	15.88
Per cent curd yield	26.37	28.25	32.00
Per cent fat in curd	18.24	20.41	18.88
Per cent solids in curd	36.79	41.26	39.19
Per cent whey yield	70.30	69.00	67.82
Per cent fat in whey	0.98	0.11	0.08
Per cent solids in whey	8.34	7.73	7.89
Cheese texture (Av.)	Coarse	Medium smooth	Medium smooth
Cheese flavor (Av.)	Clean	Clean	Clean
Cheese flavor (after 15 days at 40°F.) (Av.)	Clean-acid	Clean	Clean

All milk passed through the homogenizer at 130°F. (54.4°C.) unless otherwise designated.

The major points of interest can be easily followed in the recordings of table 1.

Table 2 has been prepared to show further the variations due to homogenization. In this table the results represent the averages of 18 batches of cream cheese prepared to study homogenization influences.

Of particular interest were the studies conducted at pressures below 2500 pounds. The practical value of establishing a minimum pressure was very important. The point at which drainage was very rapid and fat losses in the whey not excessive was used as a guide in studying minimum homogenization pressures. In this relationship it was established that the beneficial influences of homogenization was completely lost at a pressure below 750 pounds. At 1500 pounds pressure the beneficial influences of homogenization were noticeable in part.

TABLE 2

Showing average of series of cream cheese prepared to study homogenization influences upon low and high fat content cream

FACTORS STUDIED	PER CENT FAT IN CREAM				
	6.06	10 88	14.12	17 47	26 50
Homogenization pressure in pounds	2500	2500	2500	2500	2500
Per cent solids in cream	15.88	19 46	22 98	25 46	No analysis
Per cent curd yield	28 25	31 00	38 00	50.60	58 60
Per cent fat in curd	20 41	37.50	No analysis	No analysis	No analysis
Per cent solids in curd	41.26	47.75	45.50	44 83	No analysis
Per cent whey yield	69 00	68.50	61 50	49.00	41 50
Per cent fat in whey	0.11	0.21	0 22	0 20	1.19
Per cent solids in whey	7.73	7.32	7.67	7 50	8 23
Cheese texture (Av.)	Medium smooth	Medium smooth	Smooth	Very smooth	Very smooth
Cheese flavor (Av.)	Clean	Clean	Clean	Clean	Clean
Cheese flavor (after 15 15 days at 40°F.) Av	Clean	Clean	Clean	Clean	Clean

Skim-milk was homogenized at low and high pressures. It was not possible through homogenization to improve the texture of a curd prepared from skimmed milk.

The endeavor to associate the variations due to homogenization with the reduced size of fat globules has been summarized in table 3. The figures show that smooth texture and reduced size of fat globules resulted from homogenization. It could not be concluded, however, that smooth texture and reduced size of fat globules due to homogenization were directly related. This point was investigated further in connection with the composition and additional homogenization trials. An attempt was made to associate smooth texture with size of fat globules, extent and size of fat clumps, and fat content of the cream in these trials which are reported under the composition studies.

The average size of fat globules was reported as 4.82 microns in diameter for the Station Jersey milk during a previous investigation.[1] The average size of fat globules was reported in this study as 3.88 microns in diameter. Although this could have been explained as a normal variation in size, it was attributed to the fact that fat globules were measured in raw milk for the first investigation. In this work the fat globule measurements were made with pasteurized milk.

TABLE 3

Showing the relationship of homogenization to fat globule size and cheese texture

FACTORS STUDIED	HOMOGENIZATION PRESSURE		
	None	2500 pounds	4500 pounds
Number of fat globule measurements averaged	500	500	500
Average size of fat globules in microns	3 88	1.56	0.97
Percentage of fat globules larger than average for normal milk used	54.20	3 40	0 00
Cheese texture	Coarse	Smooth	Very smooth

From the studies conducted it was possible to determine that a homogenization pressure of 2000 pounds or above produced the desirable smooth texture of the cheese. From a microscopic study it was possible to determine that a homogenization pressure which reduced the diameter of the fat globules 50 per cent or more was sufficient to produce the desirable influence of homogenization in producing a smooth texture in the cheese. It was also discovered to be essential to have a pressure of 2000 pounds or more in order to reduce the losses of fat in the whey.

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[1] A. C. Dahlberg and J. C. Marquardt. 1924. Filtration and Clarification of Milk. N. Y. Agr. Exp. Sta., Tech. Bul. 104.

Effect of rennet variations

Nine comparisons indicated that variations in the amounts of rennet used in milk did not materially affect the initial drainage of whey from the curd. Excessive amounts of rennet, however, had the deterimental effect of giving the drained curd a rather dry appearance and in some trials caused a lumpy curd. The tabulated averages of the rennet comparisons are given in table 4. The rennet variations reported in table 4 were held within definite limits required to form a curd from milk held at 72°F. (22.2°C.) for twelve to eighteen hours. The milk for these comparisons was homogenized at 2500 pounds pressure.

TABLE 4

Showing the results of varying the amounts of rennet in nine batches of milk

FACTORS STUDIED	RATE OF RENNET PER 1000 POUNDS OF MILK*		
	1.5 cc	20 cc.	60 cc
Per cent milk fat in milk	5.46	5.46	5.46
Per cent solids in milk	15.37	15.37	15.37
Per cent curd yield	31.75	32.75	33.40
Per cent fat in curd	18.25	18.20	18.20
Per cent solids in curd	34.08	33.91	32.43
Per cent whey yield	64.50	66.00	66.50
Per cent fat in whey	0.07	0.06	0.12
Per cent solids in whey	7.24	7.33	7.34
Cheese texture (Av.)	Smooth	Smooth	Smooth
Cheese flavor (Av.)	Clean	Clean	Clean
Cheese flavor (after 15 days at 40°F.) (Av.)	Clean	Clean	Clean

* It was noted throughout the work that very small amounts of rennet produced a satisfactory curd when set at 72°F. for twelve to eighteen hours.

It was observed that a more uniform curd was obtained in the homogenized milk than in the milk which was not homogenized.

This was true because the homogenized milk was homogeneous and allowed the culture and rennet to act equally upon all portions of the milk. The upper layer of the unhomogenized milk contained more fat than the lower layer. As a result the culture and rennet acted upon two layers of varying composition. This terminated in difficulties during the drainage process. Excessive fat losses and a cheese of uneven texture resulted. This condition was observed with milk but trials reported towards the close of this project indicated that these conditions do not hold uniformly for cream. Unhomogenized cream heated to 180°F. (82.2°C.) and held for thirty minutes produced a smooth texture cheese. The fat losses in the whey, however, were excessive. Apparently the heavy creams did not divide into distinct upper and lower layers thus causing the unusual rennet and culture action noted with milk. Likewise, the excess fat in the cream was sufficient to produce a cheese of smooth texture, regardless of heavy fat losses in the whey.

Effect of commercial culture variations

The improper use of commercial cultures in the manufacture of soft cheese proved to be the leading factor in producing off-type curds. Texture, flavor, and keeping quality were affected equally by improper use of pure cultures.

In the cream cheese investigations it was deemed desirable to add sufficient culture to the milk or cream to control flavor without the development of a strong acid reaction. Although this was a delicate point in the case of milk or cream set with rennet, it was established by using a culture that titrated 0.6 to 0.8 per cent expressed as lactic acid being titrated with a tenth normal sodium hydroxide solution. Cultures with a reaction above or below these limits were not satisfactory.

A curd with an expressed acid reaction of 0.2 to 0.5 per cent gave the most satisfactory results. Curds below this limit usually developed an off flavor, whereas higher acid curds regularly developed into a slightly sour cheese.

Curds and whey were not changed in composition as a result of culture variations. Some curds prepared without a culture

TABLE 5

Showing results when culture variations were made

(Milk was set with rennet)

FACTORS STUDIED	RATE OF CULTURE PER 1000 POUNDS OF MILK							
	None	10 cc	20 cc	25 cc.	50 cc.	100 cc	200 cc	400 cc
Per cent milk fat in milk	5.72	5 51	5.72	5 51	5 62	5.72	5.72	5 72
Per cent solids in milk .	15 34	14 15	15 34	14 15	14.79	15.44	15.44	15.34
Per cent curd yield ..	22 00	21 75	28 00	23 00	25.00	30.00	31.00	30 00
Per cent fat in curd .		22 00		21.50	20.75	18.00	18.00	
Per cent solids in curd . . .	43.46	43 94	38 34	42 47	40.30	34.80	35 26	38.57
Per cent whey yield....	74.00	78 00	69.00	77 00	73.00	65 00	64.00	68 00
Per cent fat in whey	0.12	0 19	0.11	0.20	0.13	0.08	0 05	0.09
Per cent solids in whey .	7 33	7 73	7.46	7.74	7.71			7.30
Cheese texture (Av.) . .	Coarse	Slightly coarse	Smooth	Slightly coarse	Slightly coarse	Smooth	Smooth	Smooth
Cheese flavor (Av.). . .	Bitter	Mild	Clean	Clean	Clean	Clean	Clean	Clean
Cheese flavor (after 15 days at 40°F.) (Av.)...... .	Bitter	Mild clean	Mild clean	Mild clean	Slightly sour	Sour clean	Sour	Sour

developed bitter flavors. Flavor control was possible by using 10 to 25 cc. of culture per 1000 pounds of milk. Greater amounts of culture reduced the quality of the cheese due to acid development.

The proper use of cultures improved the flavor of the curds. Cultures, however, did not influence the texture of the curds in a beneficial manner.

The studies of milk and cream of different composition indicated that an increased fat and solid content tended to inhibit the development of acid flavors.

The above comments will probably indicate more regarding the use of cultures than can be obtained from table 5.

Effect of setting temperature changes

These studies showed that a temperature from 68° to 72°F. (20° to 22.2°C.) was most desirable for a proper curd and acid formation. This temperature was selected from the consideration of developing a proper curd in twelve to eighteen hours with rennet.

The results showed that higher temperatures were very detrimental to the quality of the cheese when a proper curd was desired in twelve to eighteen hours. This was not true with cream cheese set without rennet but set with a culture only. Temperatures below 68°F. (20°C.) produced no objectionable condition in the curds. Employing a temperature below 68°F. (20°C.) had no advantage and added materially to the length of time required to form a satisfactory curd. Low temperatures of setting also introduced a factor of slow and irregular drainage of the whey from the curd.

Throughout the work the importance of maintaining definite temperature control was evident. Rising temperature in the curd during setting favored the production of a granular curd, and excess acid development. With rennet curds, the action of the rennet upon the curd was too severe and caused excessive drainage as a direct result of too high a setting temperature.

It was further noted that allowing the temperature to fall below the normal limits caused difficulties in making the cheese. At sub-normal temperatures the curds appeared to be properly firmed for heating and draining. In such cases, however, upon the application of heat, it was noticed that the curds were slightly too soft. Although this condition could be controlled to produce a favorable cheese in the majority of cases by slowing the heating process, it introduced factors which made the process very burdensome to the maker.

Composition investigations

The composition of the milk or cream constituted the first factor in determining the nature of the resulting product in soft cheese making. In cottage cheese the typical granular texture was due to a low milk fat content in the original milk used. It was recognized that an increase in milk fat or a physical treatment as homogenization tend to destroy this texture in the manufactured cream cheese.

The makers of cream cheese were confronted with the problem of obtaining a smooth texture by the addition of milk fat and the employment of physical means. The problem of determining the limitation of these methods has been considered in this work.

It has been observed that increasing the homogenization pressure decreases the loss of fat in whey. And further, that increasing the homogenization pressure hinders the separation of whey. To overcome the slow drainage the curd was heated. There was, however, a very definite point beyond which the curd could not be heated.

The viscosity of the milk or cream was affected by the temperature of homogenization.

Experimental results favored homogenization of milk at a temperature of 130° to 140°F. (54.4° to 60°C). Homogenization above these limits resulted in a reduced viscosity, and promoted difficulties in drainage. Employing temperatures below 130°F. (54.4°C.) increased the viscosity, and likewise introduced difficult problems in drainage.

Although it was more difficult to drain the whey from cream homogenized below 130°F. (54.4°C.), it was found desirable with cream of low viscosity to homogenize at temperatures as low as

TABLE 6

Showing relationsip of composition of milk and cream to cream cheese quality

FACTORS STUDIED	PER CENT OF MILK FAT IN CREAM							
	6.82	10 01	11.75	13 26	14.12	17.45	17.50	26.50
Per cent solids in cream	20.04	19.46		22.54	22.98	25.46		
Per cent solids in curd	48.37		47.75	44.83				
Per cent fat in whey	0.29	0.29	0.13	0.15	0 22	0.27	0.15	1.19
Per cent solids in whey	7.32	7.70	6.94	7.20	7.67	7.80	7.20	8.23
Cheese texture (Av.)	Granular	Slightly granular	Slightly granular	Smooth and creamy	Smooth and creamy	Very smooth	Very smooth	Very smooth
Cheese flavor (Av.)	Clean	Clean	Clean	Clean	Clean	Clean	Clean	Clean
Cheese flavor (after 15 days at 40°F.) (Av)	Clean	Clean	Clean	Clean	Clean	Clean	Clean	Clean

110°F. (43.3°C.). This introduced the factor of slow drainage, but it insured a smooth textured curd.

The physical nature and treatment of the milk or cream were considered at the same time composition studies were conducted.

The studies with milk and cream of varying percentages of milk fat showed that a cream containing from 15 to 18 per cent milk fat resulted in a smooth pleasant flavored cheese of good keeping quality. Cream containing more than 18 per cent milk fat caused excessive fat losses in the whey, and in some cases it was very difficult to obtain a proper separation of whey.

TABLE 7

Showing relationship of fat content of cream to size of fat globules and size and number of fat clumps as related to cheese texture

FACTORS STUDIED	PERCENTAGE OF FAT IN CREAM		
	8.42	13.69	17 72
Homogenization pressure in pounds	2500	2500	2500
Number of fat globule measurements averaged	200	200	200
Average size of fat globules	1.68	1.87	2.05
Number of fat clumps per 100 single fat globules counted	44	50	63
Average size of fat clumps in microns	(3.4 × 4.9)	(5 × 6.7)	(7.6 × 8.9)
Cheese texture	Coarse	Smooth	Very smooth

In table 6 are recorded the findings of varying the composition of the milk or cream. Twenty batches of cheese were studied for these comparisons.

The size of fat globules and the size of fat clumps and their number were recorded in connection with the composition studies. The data summarized in table 7 indicated that the size of fat globules and the percentage of fat present control the smooth texture of the cream cheese. The summary of table 3 shows that for milk and cream of like fat content there was an apparent direct relationship between the small fat globules due to homogenization and smooth texture of the cheese. It was not possible to associate the size of fat clumps and their number with the smooth texture of the cheese. From table 7 it was possible to deduct that the size and number of fat clumps were related in a direct proportion to the amount of fat in the cream. The factors responsible for increased clumping were not investigated as the results did not associate smooth texture with size and number of fat clumps.

Heat treatment and its relationship to the cheese

In order to aid further in the production of a smooth texture in cream cheese and to facilitate the drainage of whey with minimum fat losses, heat treatment studies were conducted. The first point studied related to the temperature of heating the cream. The second study concerned itself with the heating of the curd.

The procedure of heating the milk or cream to 145°F. (62.8°C.) and holding for thirty minutes had a marked influence upon the flavor and keeping quality of the cream cheese. It did not, however, affect the texture of the cheese. It was necessary to heat the milk or cream to 180°F. (82.2°C.) and hold for ten minutes before a noticeable change in texture occurred. It was further noted that heating cream as high as 180°F. (82.2°C.) and holding for twenty to thirty minutes improved further the texture of the cheese. Although a temperature of 180°F. (82.2°C.) held for ten minutes or less fulfilled the requirements for pasteurization regulations, it was deemed advisable to increase the holding time twenty to thirty minutes. By this practice the additional small but positive improvement in texture was obtained. These results were observed with curds that formed at 72°F. (22.2°C.) in twelve to eighteen hours.

An attempt was made to produce a cheese of smooth texture without homogenization by heating cream containing 18 per cent fat to 180°F. (82.2°C.) and holding for thirty minutes. It was possible to produce the desired smooth texture in cheese made from unhomogenized cream by pasteurizing at 180°F. (82.2°C.) and holding for thirty minutes. But the fat losses in the whey from unhomogenized cream were excessive as previously noted in table 1 relating to the observation of milk. Increasing the pasteurization temperature did not reduce the fat losses in whey drained from curds of unhomogenized cream.

High temperature pasteurization produced a soft curd and required special treatment to facilitate drainage. This in turn resulted in a cream cheese with a very plastic smooth texture. Curds that appeared very soft and mushy required heat treatment for drainage. No definite procedure or temperature could be set for this purpose. Gentle agitation and the gradual application of heat were essential for producing desirable drainage.

It was found necessary to cool the milk or cream to 130° to 140°F. (54.4° to 60°C.) before homogenization in order to control the viscosity. Cream homogenized at a low temperature was very viscous and drained slowly, as previously noted. High viscosity cream always produces a smooth curd.

The experimental results indicated that a curd heating as high as 96°F. (35.6°C.) was necessary to facilitate whey drainage. It was also determined that the time of cooking varied widely under different conditions. As a rule it required from two to four hours of cooking in order to drain the curds with a high milk fat content.

In special cases where the cream cheese was set with a culture and no rennet, it was required to increase the cooking temperature from 96°F. (35.6°C.) to as high as 110°F. (43.3°C.) before proper drainage was possible.

Cream set at 86°F. (30°C.) with normal amounts of culture and rennet required heat treatment to facilitate drainage. These curds formed in four to six hours and never required a temperature above 96°F. (35.6°C.) to facilitate drainage.

Analyses of commercial cream cheese

In an endeavor to establish the composition of commercial cream cheese several samples were purchased and analyzed. These analyses are reported in table 8. The figures in this table indicated that the desirable texture and flavor were obtained in cheese made from high fat content cream. In two cases, however, this was not true. Although samples 5 and 6 in table 8 had the proper texture, they were off-flavor and yeasty. This condition was due either to the use of low grade raw materials, storing the

TABLE 8

Showing composition of commercial cream cheese

CHEESE NUMBER	1*	2*	3	4	5	6	7	8
Fat percentage	4.50	4.78	41.00	41 00	36.00	37 35	44 82	36.91
Moisture percentage	75.92	76.07	48.06	47.00	47.76	47.52	No analysis	No analysis
Flavor	Lacking in flavor	Lacking in flavor	Sweet	Sweet	Yeasty and off flavor	Yeasty and off flavor	Sweet	Sweet
Texture	Coarse and grainy	Coarse and grainy	Smooth	Smooth	Smooth	Smooth	Very smooth	Very smooth

* Samples 1 and 2 were purchased in bulk. This cheese although sold as cream cheese resembled a very high grade cottage cheese due to its low fat content and granular texture.

cheese at a high temperature, or keeping the cheese over too long a period. The importance of using excellent raw material and not subjecting the cheese to improper temperature or holding it too long were factors essential in the production of an excellent quality cheese.

These cheese varied widely in composition, texture, and flavor. In consequence, their price also covered a wide range. Needless to say, the cheese with a very smooth slightly sticky texture demanded the highest price.

The author has also analyzed samples of cream cheese containing fats other than milk fat. Although these foreign fats produced a very smooth texture, their effect upon the flavor of the cheese was detrimental. The presence of foreign fats in cream cheese can be detected by observing the size and appearance of fat globules. This is not a perfect method of detection and cannot be relied upon in cases when the foreign fat has been homogenized. It can be employed to advantage, however, in a general study of the product with reference to the addition of foreign fats to the finished cheese and the efficiency of homogenization. After the microscopic examination chemical means were used to establish the presence of foreign fats. The addition of foreign fats to cheese is adulteration and should not be practiced.

Cream cheese methods compared

Two methods commonly employed in the manufacture of cream cheese were compared with the method developed at the Station. The first method required 180°F. (82.2°C.) for thirty minutes for pasteurization. The cream was homogenized at 140°F. (60°C.) and 1800 pounds pressure. The cream was set at 75°F. (23.9°C.) with 1 cc. of rennet per 600 pounds of cream. The following day, when the soft jelly-like curd was formed, the curd was gently stirred and heated from 94° to 96.°F. (35° to 35.6°C.). Rivulets of whey on the stirring rod dipped into the curd indicated that the cooking was sufficient. At this point the curd was cooled to 50°F. (10°C.) and after an addition of 1 per cent salt, the curd was drained in bags.

The second method varied from the above method in that the cream was set at 90°F. (32.2°C.) with 0.25 per cent of culture without the addition of rennet. This method required cooking at 110°F. (43.3°C.) in order to facilitate proper drainage.

The method developed at the Station required a cream containing 15 to 18 per cent and pasteurization at 180°F. (82.2°C.) for thirty minutes. The cream was homogenized at 110°F. (43.3°C.) and 2500 pounds pressure. The cream was set at 72°F. (22.2°C.) with 10 to 25 cc. of culture and 1.5 to 20 cc. of rennet per 1000 pounds of cream. The condition of the milk determined the amount of culture and rennet used. After twelve to eighteen hours, according to the appearance of the curd, the curd was heated until ready for drainage. This heating required two to four hours, and a temperature varying from 94° to 110°F. (34.4° to 43.3°C.), depending upon the nature of the curd. Fine salt was incorporated in the drained curd at the rate of 1¼ pounds per 100 pounds of curd.

The cream cheese made by the three methods was smooth in texture. It was necessary to control carefully the acid development in the cheese set with a culture at a high temperature. Failure to do so resulted in a high acid curd of granular texture.

The cheese set with rennet only developed a mild sweet flavor. The cheese made by the other methods developed a mild flavor that was distinctly different from the cheese made from cream set with rennet only.

The practice in the first method described of cooling the cream to 50°F. (10°C.) before drainage was an added precaution to prevent fat losses in the whey. It was also an added precaution to prevent further acid and off-flavor development. The desirability of following this procedure was governed largely by the condition of the raw material and the facilities of cooling rapidly and conveniently.

According to Fisk,[2] the practice in the first method described of adding 1 per cent of salt before draining was a precaution to prevent further acid development in the curd.

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[2] The information regarding the addition of salt before drainage and cold drainage was obtained through the courtesy of Prof. Walter W. Fisk, Cornell University, Ithaca, N. Y. It was also through the courtesy of Professor Fisk that the information regarding the two commonly used methods for making cream cheese was obtained.

Experiments conducted at the Station established this point by showing that the addition of salt hindered the development of lactic acid in milk. Only 2 per cent of salt was necessary to check the acid development in milk to which a culture had been added. The milk treated with salt and incubated at 72°F. (22.2°C.) for eighteen hours titrated less than 0.25 per cent expressed as lactic acid and titrated with a tenth normal sodium hydroxide solution. The control titrated over 0.8 per cent with the same method of titration. Salt added to the control after the acid development had taken place did not alter the titration. The action of salt in checking acid development was further established by titrating the whey draining from salted and unsalted curds.

It was necessary to use a fine salt when salting the drained curd according to the method developed at the Station. Both methods were satisfactory for incorporating salt in the curd. Excellent results were obtained by salting the drained curds when high grade raw materials were used. Salting before draining was found desirable when curds from inferior raw material were prepared.

DISCUSSION

The quality of soft cheese varieties can be improved by homogenization and high pasteurization temperatures of the milk or cream.

The texture of soft cheese can be improved by homogenization. Homogenization also reduces the fat losses in the whey. To improve the texture of the soft cheese and minimize the fat losses in the whey an homogenization pressure of 2000 pounds or more is required. Or, conversely, a pressure sufficient to reduce the diameter of the fat globules 50 per cent or more will aid in the production of the desired texture and minimize fat losses in the whey.

Homogenization at a pressure of 4000 pounds or above further improves the texture of the cheese. It does not reduce the fat losses in whey more than a pressure of 2000 pounds. Difficulties are encountered in attempting to drain curds produced from milk or cream homogenized above 4000 pounds pressure.

Homogenization of milk at a temperature of 130° to 140°F. (54.4 to 60°C.) is essential to eliminate viscosity variations produced at higher or lower temperatures which interfere with the proper drainage of the curd. Homogenization of cream at 110°F. (43.3°C.) is desirable to insure a smooth textured cheese. By increasing the viscosity of the cream, this homogenization temperature introduces the factor of slow drainage which is compensated by the assurance of a smooth curd.

It is not possible to conclude that the smooth texture of soft cheese is directly related to the small sized fat globules resulting from homogenization. Nor is it possible to associate the size and number of fat clumps resulting from homogenization directly with the smooth texture of the resulting cheese. Cream cheese made from cream containing high percentages of fat is smoother in texture than a cheese made from a thinner cream, due to the additional fat. This is true regardless of the size of fat globule clumps and their number.

Rennet variations influence the quality of soft cheese. Excessive amounts of rennet result in a dry-appearing cheese. Rennet action is more uniform on homogenized than on unhomogenized milk. This is not uniformly true with cream. Cream cheese set with rennet requires sufficient rennet to form a soft jelly-like curd at 72°F. (22.2°C.) in twelve to eighteen hours. One and five-tenths to 20 cc. of rennet per 1000 pounds of milk to which a culture is added is sufficient to produce the desired curd. This curd is formed in four to six hours when the setting temperature is raised to 86°F. (30°C.) and the maximum amount of rennet used.

Neufchatel and cream cheese flavors are improved by the proper use of a commercial culture. Small quantities of culture are used to control off-flavor developments. Ten to 25 cc. of culture per 1000 pounds of milk are required to control flavors in soft cheese. The use of excessive amounts of culture is deterimental, resulting in the development of sour curd, and also a granular condition of the curd. Cultures properly used have no effect upon the curd texture. It was observed, however, that certain proportions of culture and rennet were responsible for a granular condition in the curd.

Neufchatel and cream cheese are set at 68° to 72°F. (20° to 22.2°C.) to produce the desirable curds in 12 to 18 hours. Temperatures below 68°F. (20°C.) introduce subsequent drainage difficulties. Temperatures above 72°F. (22.2°C.) result in granular and sour curds, when set twelve to eighteen hours.

The fat content of milk or cream influences the texture and flavor of soft cheese. Fat content and homogenization are closely associated in controlling soft cheese texture. Cottage cheese is granular in texture, and this texture cannot be made smooth by homogenization, due to the lack of fat in the skimmed milk used.

Neufchatel cheese texture is improved by homogenization of the milk. Homogenization of milk reduced the fat losses in the whey.

A very desirable texture cream cheese results from high temperature pasteurization and homogenization of cream containing 15 to 18 per cent fat.

Pasteurization of milk or cream at 145°F. (62.8°C.) and holding for thirty minutes improves the flavor and keeping quality of soft cheese. Pasteurization of milk or cream at 180°F. (82.2°C.) and holding for ten to thirty minutes improves the flavor and keeping quality of soft cheese. This high temperature also has a beneficial influence of producing a smooth texture in the cheese at 72°F. (22.2°C.) for twelve to eighteen hours. It is possible with cream containing 18 per cent fat by pasteurizing at 180°F. (82.2°C.) and holding for thirty minutes to produce a smooth-textured cheese. This texture is equal in smoothness to a cheese prepared from homogenized cream. The unhomogenized cream subjected to a high pasteurization temperature caused excessive fat losses in the whey.

Cream cheese prepared from cream containing 15 per cent or more fat set with culture and rennet, or with rennet only, requires special curd heating for drainage. It is necessary to heat the curd to 94° to 96°F. (34.4° to 35.6°C.) during a period of two to four hours to facilitate proper drainage. Cream cheese prepared from cream containing 15 per cent more fat and set with a culture required a curd heating as high as 110°F. (43.3°C.) to facilitate proper drainage.

Commercial brands of cream cheese usually contain 48 per cent moisture and 40 per cent fat. Foreign fats in cream cheese and the efficiency of homogenization are studied by the aid of the microscope. Chemical analyses are required to establish definitely the presence of foreign fat in cream cheese.

Cream cheese was made successfully by three methods. One method required rennet, the second method required a culture, while the third method, developed at the Station, required a culture and rennet for setting the cream.

For comparative purposes it was not found advantageous to drain the curds under pressure. Consequently, the yields and composition were not comparable to commercial cheese.

Setting cream cheese with rennet only required absolute temperature control for the development of a proper curd. Cream cheese set with a culture developed a granular texture only when the acid development was high. The combined use of a culture and rennet gave the most satisfactory results in the formation of uniform curds. Cream cheese set with rennet only developed a mild sweet flavor. Cream cheese set with a culture, or with a culture and rennet, developed a mild flavor. This flavor was distinctly different from the flavor of the cheese set with rennet only.

Cooling the curd to a temperature of 50°F. (10°C.) before draining was an added precaution to prevent fat losses in the whey and the development of sour and off-flavors.

The curd was successfully salted before or after drainage. The object of salting the curd before drainage was to check acid development. It was possible to salt drained curd successfully by using a fine salt according to the findings at the Station.

SOME OBSERVATIONS ON THE FREEZING POINTS OF VARIOUS CHEESES*

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Inquiries have reached this bureau relative to the freezing point of cheese, which, aside from its scientific importance, is of practical interest, particularly with regard to the storage and transportation of cheese in commerce. A search of the literature has revealed no information on this subject; consequently, some measurements have been made in this laboratory, the results of which are given below. Since the time available for this work was limited, only a small number of observations were possible, but it is thought that they are accurate and comprehensive enough to be of practical value. It may be said that the freezing point will vary with different specimens of the same kind of cheese, depending upon the moisture content and degree of ripening.

Stiles (1) and Carrick (2) have discussed the theory of freezing and the potentiometric determination of the freezing point of various foods.

APPARATUS

The temperature was measured electrometrically by the use of standardized thermocouples with the cold or reference junction at 0°C. These thermocouples were made of No. 26, B. & S. gauge, copper-constantan wires. A Leeds and Northrup Type K potentiometer was used in connection with a Type R galvanometer placed in a Julius suspension. A compartment kept at a temperature of approximately −18°C. served as the cold air bath freezing chamber.

EXPERIMENTAL

Small blocks cut from near the center of the cheese were used as samples. The bare metal thermocouples were buried in the

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center of each block about an inch from the surface. On account of the eyes in the Swiss cheese a sample was either ground fine and then compressed, or it was cut in halves, which were clamped together after the junction had been carefully placed between them. The samples were electrically insulated from each other and the ground by placing them upon glass plates in the freezing chamber. Readings of the potential were taken every five minutes until freezing began. After that time they were taken at one or two minute intervals. Observations were made upon a number of cheeses during the thawing of the sample. Moisture determinations were also made upon a few of the cheeses.

TABLE 1

Shows freezing point and moisture content of standard varieties of cheese

NAME OF CHEESE*	FREEZING POINT	MOISTURE CONTENT
	°C.	per cent
Cottage	−1.2	78.7
Cheddar (processed)	−6.9	38.8
Limberger	−7.4†	44.4
Picnic Swiss (processed)	−8.1	
Brick (processed)	−8.7	
Swiss (imported)	−9.6†	
Swiss	−10.0‡	34.4
Cheddar	−12.9	33.8
Roquefort	−16.3†	39.2

* The cheeses were all domestic with the exception of the imported Swiss.

† These were checked by thawing curve values which did not differ from them by more that 0.2°C.

‡ This is the average of three freezing and two thawing curve values, which did not vary more than 0.3°C. Only one of these curves is shown in figure 1.

Data obtained from these observations are given in table 1 and the relation between the cooling curves of a number of standard varieties of cheese is shown in figure 1.

DISCUSSION

It is known that supercooling, or the lowering of the temperature below the freezing point without ice formation, occurs in many substances. A little supercooling is desirable in order to show the subsequent rise to the equilibrium point and to give positive evidence of the initial ice formation. However, this supercooling effect and the marked lag in the rise in temperature following the initial solidification result in errors, the magnitude of which depends upon several factors, such as the size of the samples and their constitution.

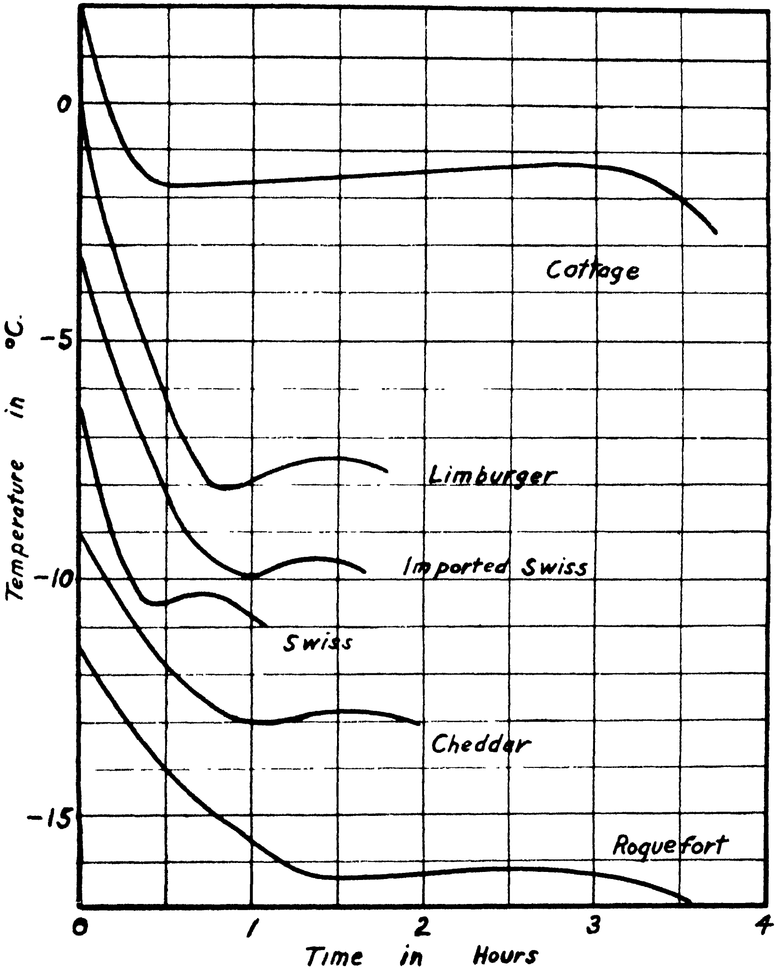

FIG. 1 FREEZING CURVES OF VARIOUS CHEESES

When solutions are supercooled the ice which finally separates as pure solvent decreases the volume of the remaining solvent, thereby increasing the concentration. In thawing, however, this process is reversed. For this reason freezing points determined by supercooling are likely to be lower than the true values, while those determined from the break in the thawing curves tend to be higher. Therefore, a close agreement between measurements made by both methods serves to confirm their accuracy.

The fact that the supercooling which occurred was not excessive and the values were only about 0.2°C. lower than those from the thawing curves indicated that the errors were negligible. Therefore, no corrections were applied to the freezing points which were taken from the highest point on each curve on the rise after supercooling.

The low freezing point of the Roquefort cheese is probably due to its relatively high salt content.

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(2) Carrick, D. B.: Cornell Agr. Exp. Sta. Mem. 81, December, 1924.

THE EFFECT OF HEAT TREATMENT OF SKIM MILK UPON THE BAKING QUALITY OF THE EVAPORATED AND DRIED PRODUCTS*

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It is well known that the heat treatment to which a raw milk is subjected affects the stability of its evaporated product to sterilization temperatures. This fact is made use of in the evaporated milk industry, where maximum stability and good body of the products are desirable qualities. In the case of condensed milks it has been found that "thickening" is favored if temperatures of forewarming near the boiling point are used.[1] The phenomena which are concerned in these cases are probably the result of a change in the degree of hydration and in the degree of dispersion of the proteins in the products.

With these facts in mind, as well as those relating to flour strength, it seems reasonable to suppose that the degrees of hydration and dispersion of the proteins, as indicated by the consistency or body of a product, might affect the property of a bread dough mix in baking.

In addition to the heat treatment in the raw state, milk used in the manufacture of dry milk has the possibility of being further affected by the temperatures to which it is subjected in the manufacturing process.

EXPERIMENTAL

Several preliminary experiments indicated that the water-holding capacity of the constituents of milk, as indicated by the body or viscosity of their solution, was not changed when the product was converted into the dry form.

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* Received for publication November 18, 1927.

[1] Leighton, A., and Deysher, E. F. Proc. World's Dairy Congress, ii, p.1276–84, (1923).

Two commercial samples of dry skim mik were selected which were known to have received different heat treatments before being reduced to a dry state. Samples of these dry milks were incorporated in bread dough mixes. Throughout the work the following formula was used in all baking tests:

	grams
Flour	325
Sugar	10
Salt	5
Fat	7
Yeast	10
Dry skim milk (4 per cent of weight of flour)	13
Water—as per absorption	

Experiments to determine the correct amount of water to be used in each case revealed the fact that dry skim milk made from milk which had received the higher heat treatment (sample no. 2 in table 1) seemed to impart to the dough a greater water-holding capacity than when made from milk which had received the lower heat treatment (sample no. 1). Experiments recorded in table 1 indicate that the dough made from sample no. 2 yielded a loaf of greater volume and of better texture than that made from sample no. 1, when the water content was optimum for baking purposes in each case.

TABLE 1

The effect of adding varying amounts of water to dough mixes containing different dry skim milks

SAMPLE NO 1			SAMPLE NO 2		
Baking number	Water	Volume of loaf	Baking number	Water	Volume of loaf
	cc			*cc*	
1	195	1,885	1	195	1,815
2	200	1,870	2	200	1,925
3	205	1,920	3	205	2,130
	213	Not feasible	4	213	2,085

The dough mix containing a dry skim milk of low heat treatment to which had been added 213 cc. of water was not feasible for baking because of its consistency.

After determining the optimum quantity of water to use with each dry milk, five bakings were made to determine the different heat treatments of the milk upon volume and texture of the loaf. Table 2 gives the results of these bakings.

TABLE 2

A comparison of the baking quality of dough mixes in which different dry skim milks were used

CHECK—NO DRY SKIM MILK ADDED				NO 1—DRY SKIM MILK NO 1* ADDED			NO 2—DRY SKIM MILK NO 2† ADDED		
Baking number	Volume of loaf	Weight	Texture	Volume of loaf	Weight	Texture	Volume of loaf	Weight	Texture
	cc	*grams*		*cc*	*grams*		*cc*	*grams*	
1	2,000	477	Good	1,865	496	Good+	2,085	496	Good+
2	2,050	475	Good+	1,940	494	Good+	2,130	494	Good
3	2,020	479	Good+	1,920	497	Good−	2,085	491	Good+
4	2,010	477	Good+	1,850	491	Good+	2,055	496	Good
5	1,990	473	Good	1,885	496	Good−	2,110	503	Good+

* Received a relatively low heat treatment in the manufacture.

† Received the higher heat treatment in the manufacture.

In this table are given also the results of check bakings wherein no dry skim milks had been incorporated into the dough mixes. Figure 1 shows the increase in loaf volume and in break and shred of the loaf when sample no. 2 is used.

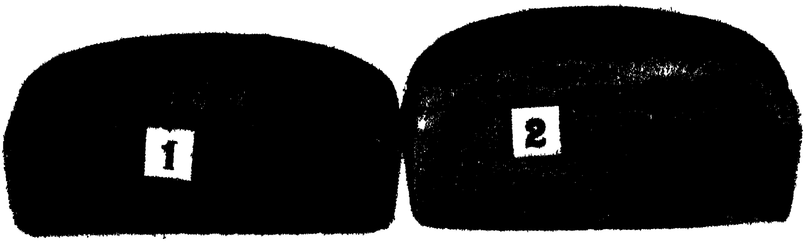

FIG. 1. SHOWING THE BREAK AND SHRED AND RELATIVE VOLUME INCREASE OF LOAF WHEN SAMPLE NO. 2 IS USED

The addition of dry skim milk from sample no. 1 has no material beneficial effect upon the loaf except to increase its weight over that of the check loaf. On the contrary, there seems to be a slight binding action when sample no. 1 is used. The weights of the loaves containing samples no. 1 and 2 are approximately equal.

The results reported in table 2 are representative of those obtained in a number of bakings in which various dry milks were used. They also show that the conditions to which milk is subjected prior to and in the manufacturing process affects the quality of its powder for baking purposes.

The variation in the water-holding capacities of dough mixes containing different dry milks (table 1) should manifest itself in the viscosity or body of a solution of the dry milk. Forty per cent solutions were therefore prepared and their relative viscosities determined,[2] as shown in table 3.

TABLE 3

Showing the relative viscosities of forty per cent solutions of the samples of dry skim milks used in baking tests (table 2)

SAMPLE NO. 1	SAMPLE NO. 2
2.50–3.00	25.0–33.0

In the same concentration of solids sample no. 2 shows a marked increase in viscosity over that of sample no. 1.

In order to ascertain the relative values of various temperatures of heat treatment and to correlate heat treatment of a milk, viscosity of its concentrated solution and baking quality, fresh skim milk was heated in a water bath for different lengths of time at the chosen temperatures and condensed to approximately 40 per cent solids. Relative viscosities were determined upon each of the samples at 40 per cent, 30 per cent, and 20 per cent concentration of solids. The results are plotted in figure 2.

The results indicate that heating a milk for a short time at

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[2] The values given in table 3, and plotted in figures 2 and 3, were obtained with a viscosimeter of the Ostwald type, and hence the term "relative viscosities" is used. The shape of the curves indicate, however, that structural properties are dealt with at the higher concentration and therefore properties of plastic flow are concerned.

85° or 95°C. has a marked effect upon the body of the product when reduced to 40 per cent solids. The effect is not so noticeable at 75°C. To obtain a marked increase in viscosity the time of heating at this temperature seems to be about four hours. A temperature of 65°C. apparently has little effect upon the viscosity even after four hours of heating. The shapes of the curves for the different temperatures readily explain the reason

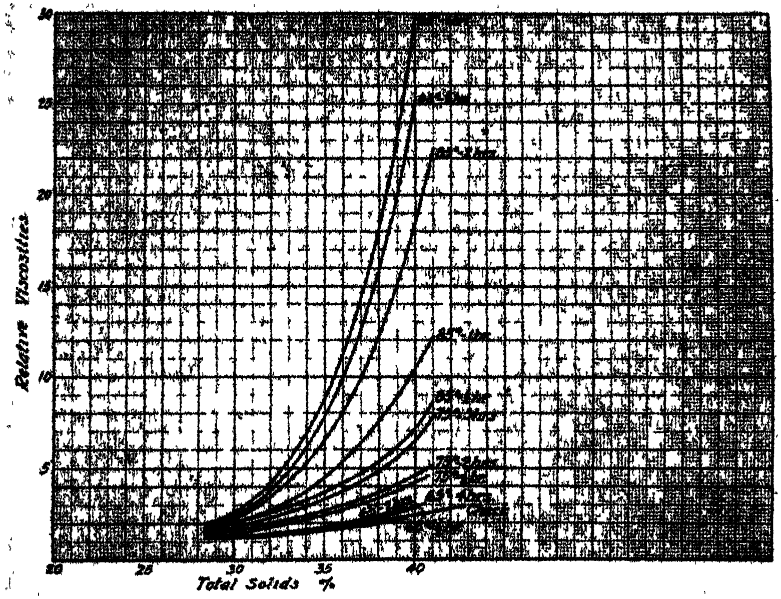

FIG. 2. SHOWING THE RELATIVE VISCOSITIES OF VARIOUS CONCENTRATIONS OF SKIM MILKS WHICH HAD BEEN HEATED AT DIFFERENT TEMPERATURES FOR VARYING PERIODS OF TIME

for wide differences in the bodies of 40 per cent solutions of milk heated at various temperatures. The change in the body of the product with increase in the temperature of heating is abrupt. The relative effects of these temperatures are better shown in figure 3 where the relative viscosities of 40 per cent solutions obtained for each temperature are plotted against the time of heating.

Table 4 gives the results of baking tests upon the various

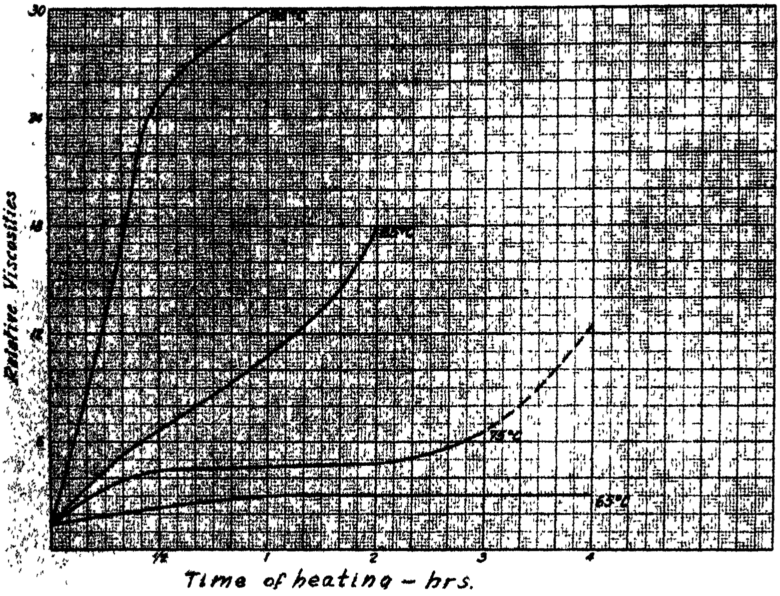

FIG 3. SHOWING THE RELATIVE EFFECTS OF VARIOUS TEMPERATURES AND TIME OF HEATING UPON THE RELATIVE VISCOSITIES OF CONCENTRATED SKIM MILKS OF 40 PER CENT SOLIDS CONTENT

TABLE 4

Changes in baking quality due to variations in heat treatment of the milk used

TEMPERATURE	VOLUME OF LOAF				
	Time of heating				
	½ hour	1 hour	2 hours	3 hours	4 hours
	cc	cc	cc	cc	cc
65°C. (149°F.)	1,918		1,853		1,854
75°C. (167°F.)	1,942*	2,020	1,969*		2,091
85°C. (185°F.)	1,973	2,027	2,043	2,030	
95°C. (203°F.)	2,037	2,037	2,023		

* Different flour used.

samples prepared and used in the experiments reported in figures 2 and 3. The product was used in amounts equivalent to 4 per cent, of the weight of the flour, on the dry basis. This table indicates a definite correlation between the viscosity of a 40 per cent solution of a dry skim milk and its baking quality. Heating at 65°C. for four hours changed the viscosity of a 40 per cent solution only slightly (fig. 2). Very little change was noted in baking quality (table 4). A temperature of 75°C. produced a change in viscosity when continued for four hours. This sample also produced improved baking quality when added to the mix. Temperatures of 85°C. and 95°C. for shorter periods of time produced high viscosities in 40 per cent solution of the samples with corresponding improvement in baking quality.

Though only volume of loaf is recorded here it may be stated that improvement in texture was always noted with increase in volume. The weights of the loaves remained approximately constant.

To test the feasibility of viscosity of a 40 per cent solution of a powder as a criterion of baking quality several commercial samples of spray powders were chosen, the viscosities of 40 per cent solutions (dry basis) were determined, and baking tests were made.

In general it may be stated that a powder producing a 40 per cent solution of high viscosity seems to yield a better loaf than does one producing a low viscosity solution at that concentration.

DISCUSSION

During the progress of the work it was observed that various flours responded differently to the addition of dry skim milk. The increase in volume of loaf over that obtained in bakings where no skim milk was used was not appreciable in some cases, though the texture seemed improved. The problem with which we are concerned, however, was not that of increase of loaf volume and improvement of texture over that obtained in experiments where no skim milk was added, but was rather the variations in properties caused by additions of skim milks receiving different treatments in the manufacturing processes. The flours used, therefore, were those which showed the greatest variations. The problem of increased yield and adaptability of flours is one that is beyond the scope of this work.

Results of experiments indicate that the method of treatment of a skim milk has a marked effect upon the property of its powder to influence the baking quality of a bread dough mix.

The data presented upon the viscosity of milks heated at various temperatures, as well as the facts relating to the "thickening" of condensed milks, show that the changes in the milk caused by heat have an effect similar to that of an increased hydration of the proteins. The correlation between the changes in the body of the solution of the milk used and the results of baking tests seems to show that the magnitude of this physical property, obtained under certain conditions, is a criterion of whether or not a skim milk will have a material beneficial effect upon the baking quality of a dough mix into which it has been incorporated

Results given in table 2 show that the milk which had received a low heat treatment (sample no. 1) seemed to have a slight binding effect upon the dough in baking.

The temperature to which this milk had been subjected in the manufacturing process were not sufficiently great to coagulate the albumin; hence it seems probable that the binding action may be attributed to this constituent. Preliminary experiments seem to verify the correctness of this idea.

ACKNOWLEDGMENT

The baking tests presented in this paper were carried on in the laboratories of the Bureau of Chemistry under the supervision of L. H. Bailey of that bureau. We are indebted to the Bureau of Chemistry and especially to Mr. Bailey for the courtesy and cooperation which made these experiments possible.

THE RATE OF ACID PRODUCTION IN HEATED MILK*

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Extensive data on the effect of heating on acid formation in skim milk have been obtained, and plots of these data at various temperatures have been published.[1] For practical use it has been desirable to combine these curves of acid production against time at a single temperature into a single curve of time rate of acid production against temperature of heating. By interpolation from this curve may be determined the rate of acid production at any intermediate temperature and thence may be calculated actual concentrations of acid formed at this temperature over a given time. It is with the hope that other workers may be saved the necessity of accumulating and arranging similar data that this curve is published herewith.

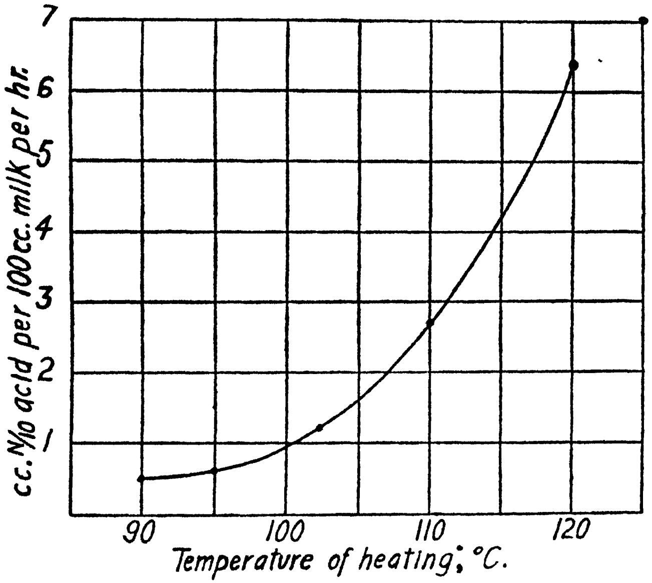

FIG. 1.—RELATIONSHIP BETWEEN RATE OF ACID FORMATION IN MILK AND TEMPERATURE OF HEATING.

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* Received for publication January 5, 1927.

[1] Whittier, E. O., and Benton, A. G., Jour. Dairy. Sci., 1927, x, 126.

INHERITANCE OF BUTTERFAT PERCENTAGE IN JERSEY COWS*

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For many years milk of the different dairy breeds has been advertised as containing a certain definite percentage of butterfat. The American Jersey Cattle Club has proclaimed that the test of Jersey milk averages approximately 5.36 per cent. That this percentage varies only slightly with age, is proved by a study of all the 365-day records completed to January 1, 1926.

The records that have been completed each year since the adoption of the Register of Merit indicate that over a period of over twenty-three years the fat percentage of Jersey milk has remained quite constant. This is shown in table 2 which gives the butterfat percentage and total fat production for the first six years after the adoption of the Register of Merit and for the last four years.

The average percentage of all the yearly tests completed is 5.365 per cent, which means that there were many records averaging lower than this, and of course, many more with higher tests. The increase in total yearly fat production indicates that breeders have constantly striven for increased yields. Their success is evident. It is also shown that the greater fat production has been accomplished only by increasing the milk producing capacity of the Jersey cow. This may be accounted for in two ways; either there is less variability in the percentage of fat than in the milk yield, or it is because dairymen have heretofore paid little attention to the butterfat percentage in their breeding operations. If the percentage of fat in milk is a definite character, inherited separately and independently of other factors, it would appear that breeders were neglecting to use an important tool for the improvement of dairy production.

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* Received for publication March 11, 1927.

Roberts, (1) in an investigation conducted in 1918, found a negative correlation between the percentage and milk yield of Jerseys, Guernseys and Holsteins. For the Jersey breed he reported a correlation of −0.354±0.013. This was a more pronounced negative correlation than for the Guernsey and Holstein

TABLE 1

AGE	NUMBER OF 365-DAY RECORDS	AVERAGE FAT PERCENTAGE
		per cent
Yearling	1,365	5.433
Jr. 2	4,091	5.437
Sr. 2	1,901	5.469
Jr. 3	2,010	5.427
Sr. 3	1,550	5.424
Jr. 4	1,537	5.417
Sr. 4	1,291	5.374
5 years	2,256	5.321
6 years	1,653	5.253
7 years	1,226	5.265
8 years	802	5.248
9 years	511	5.215
10 years	296	5.182
11 years	169	5.163
12–23 years	201	5.273
All ages	20,859	5.365

TABLE 2

YEAR	NUMBER OF 365-DAY TESTS	AVERAGE YEARLY BUTTERFAT	AVERAGE YEARLY FAT PERCENTAGE
		pounds	*per cent*
1904	18	348.61	5.402
1905	48	399.02	5.451
1906	78	403.53	5.315
1907	92	433.71	5.482
1908	103	430.83	5.441
1909	173	425.42	5.430
1922	2431	487.34	5.368
1923	1979	512.32	5.392
1924	1498	519.99	5.375
1925	1191	529.94	5.362

breeds. Wilson (2) however, states that the characters of fat percentage and milk yield are independent and have no effective influence upon each other. Graves (3), from a study of Holstein sires, also concluded that in Holstein cattle the percentage of

TABLE 3

Analysis of records with high fat percentages

Test of 80 cows with high tests who have tested dams	6.958 %
Test of 80 dams of cows who have high tests	5.952 %
Difference	1.006 %
Test of 63 cows with high tests who have tested sisters out of same dams	6.988 %
Test of 63 maternal half sisters of 63 high testing cows	5.834 %
Difference	1.154 %
Test of 41 high testing cows who have both tested dams and tested maternal half sisters	6.985 %
Test of 41 dams of above high test cows	5.885 %
Difference	1.100 %
Test of 41 maternal half sisters of above high testing cows who also have tested dams	5 844 %
Difference	1 141 %
Test of 59 high testing cows who have tested paternal grandams	6 984 %
Test of 59 paternal grandams of high testing cows	5.626 %
Difference	1.1358%
Test of 141 high testing cows who have tested half sisters by same sire	6.952 %
Average test of half sisters (by same sire) of high testing cows	5.893 %
Difference	1.059 %
Test of 53 high testing cows who have tested daughters	6.965 %
Test of 53 daughters of high testing cows	6 105 %
Difference	0.860 %
Test of 6 high test cows with proven sons	6 90 %
Test of daughters of 6 proven sons of high test cows	5.78 %
Difference	1.12 %
Average fat yield of 160 high testing cows	494.8 pounds

butterfat and milk yield seem to be inherited separately. His data also indicated that both the sire and dam contribute to the inheritance of the fat test of their daughters and that improvement in yield of butterfat can be brought about by selection for both milk yield and percentage of fat.

It was with the object of continuing this work previously begun by others, and possibly throwing more light on the subject of inheritance of butterfat percentage, that the following study was made.

TABLE 4

Analysis of records with low fat percentages

Test of 44 cows with low test who have tested dams	4.133%
Test of 44 dams of cows who have low tests	4.812%
Difference	0.679%
Test of 41 cows with low tests who have tested sisters out of same dams	4.121%
Test of 41 maternal half sisters out of low testing cows	5.035%
Difference	0.914%
Test of 26 low testing cows who have both tested dams and tested maternal half-sisters	4.140%
Test of 26 dams of above low test cows	4.761%
Difference	0.621%
Test of 26 maternal half-sisters of low test cows	4.905%
Difference	0.765%
Test of 42 low testing cows who have tested paternal grandams	4.130%
Test of 42 paternal grandams of low test cows	4.990%
Difference	0.860%
Test of 111 low testing cows who have tested sisters by same sire	4.168%
Average test of half-sisters (by same sire) of low test cows	4.660
Difference	0.492%
Test of 46 low testing cows who have tested daughters	4.167%
Test of 46 tested daughters of low testing cows	4.910%
Difference	0.743%
Test of 5 low test cows with proven sons	4.19 %
Test of daughters of 5 proven sons of low test cows	5.14 %
Difference	0.95 %
Average fat yield of 135 low testing cows	425.1 pounds

DATA PRESENTED

A study of all the records in the Consolidated Register of Merit volume revealed that of the 21,000 cows listed, 160 had completed long time tests averaging above 6.75 per cent in butterfat. Fifty-three of these records averaged higher than 7.00 per cent. The average percentage of the tested dams of these 160 cows was determined, and also the fat percentage of their tested maternal and paternal half-sisters. Likewise the yearly fat percentage of the tested daughters of these high testing cows was looked up. Proven sons of these high testing cows were included and the tests of their daughters recorded.

A similar study showed that 135 Jersey cows had completed tests with yearly average butterfat percentages lower than 4.25 per cent. Tests of their dams, sisters and daughters were determined exactly as described for the cows testing above 6.75 per cent.

Of course in a considerable number of cases, either the dam, sisters or daughters were not tested. This reduces the number of animals that can be used in the final analysis. The average results of both these tabulations are presented in tables 3 and 4.

In analyzing these studies too much emphasis should not be given to the final average percentages found. The uniformity of the inheritance of high or low butterfat tests and the amount of variation are important items that cannot be seen in the tables. The averages obtained do show that there is a noticeable tendency for the butterfat percentages to approach the average of the breed. Daughters of extremely high or low testing cows rarely tested as high or as low as their dams. Of the high testing cows there were only ten having tested dams with percentages below the breed average. The average tests of the paternal half-sisters of cows out of these ten dams all exceed the breed average by a considerable amount. In fact, the average tests of the daughters of seven of the ten sires are above 6.00 per cent. This would indicate that the cows with high tests out of average testing dams inherited their fat percentage from their sires. Fifteen of the low testing cows are out of dams with percentages above 5.00, yet in each of these cases the sires of these fifteen cows transmitted especially low tests to all their daughters.

It is significant that as far as known, every cow with a high test was either out of a dam with a percentage considerably above the average for the breed, or else was sired by a bull who transmitted high tests to the majority of his daughters. Similarly, the low testing cows were in every case either out of a low testing dam or by a sire whose daughters were low testers.

There were seven cases of mating a dam testing above 6.00 per cent with a sire all of whose daughters averaged over 6.00 per cent. In each of these cases the daughter averaged above 6.87 and four were above 7.00 per cent.

From the above lists two nationally known sires were chosen for further study. One of these bulls (A) has 20 tested daughters who have an average butterfat percentage on yearly test of 6.57 per cent. Considering the number of daughters, this bull leads the breed in the high tests of his offspring. The other sire (B) equally well known, has 34 Register of Merit daughters with an average butterfat percentage of only 4.71. It happened that the dam of each of the twentv daughters of A was tested. The average test of the dams was 5.72 per cent. As the dams were high testers the bull must have been remarkably prepotent to increase the test an average of 0.85 per cent. This bull has two proven sons whose daughters average 6.63 and 5.54 per cent respectively.

Twenty-five of the Register of Merit daughters of B were out of tested dams. The twenty-five daughters averaged 4.68 while their dams averaged 5.13. This again illustrates a bull especially prepotent except that he transmitted low tests to all of his daughters. Only one daughter did he sire that tested above the average for the breed. B has eight proven sons and each inherited the factor for low tests from their sire. Daughters of these proven sons averaged but 4.98 per cent of fat.

As these two sires were so remarkably prepotent, a study was made of their pedigrees. These are given in skeleton form and explain in a large degree the transmitting ability of both sires. The pedigree of A is a good illustration of the continuous mating of high testing animals with the result that a bull was obtained remarkably prepotent in transmitting high tests to his offspring. In total fat production, the daughters of A averaged 829.6 pounds of fat when calculated to a mature 365 day equivalent, while their dams averaged 752.4 pounds.

(A)
- Sire.............. 21 daughters Av. 5.96
  - *Paternal grandsire* 51 daughters Av. 5.63
  - *Paternal grandam* Test 6.20
- Dam............. Tests av. 5.56
  - *Maternal grandsire* 51 daughters Av. 5.63
  - *Maternal grandam* Test 5.91

(B)
- Sire.. ... . 36 daughters Av. 5.13
  - *Paternal grandsire* 18 daughters Av. 5.21
  - *Paternal grandam* Not tested
- Dam............. Test 4.18
  - *Maternal grandsire* 27 daughters Av. 4.94
  - *Maternal grandam* Not tested

To study further the relation between butterfat percentages and total fat and milk production, all of the 365-day records in the Consolidated Volume made by mature cows from five to eight years of age, were arranged in columns according to the test percentages. There were fourteen groups ranging from the lowest tests to the highest. The average fat and milk production of each group is shown in table 5.

All records exceeding 700 pounds of fat were arranged in groups and the average fat percentage of each group determined. These are listed in table 6.

There were 393 yearly records completed during 1926 by cows over five years of age. Table 7 shows the degree of correlation

TABLE 5

Classification of all 365-day records in Consolidated R. of M. Volume made by cows from 5 to 8 years of age

AVERAGE YEARLY FAT PERCENTAGE	NUMBER OF COWS IN EACH GROUP	AVERAGE FAT YIELD	AVERAGE MILK YIELD
per cent		*pounds*	*pounds*
3.50–4.20	22	492.1	12,079
4.21–4.40	80	487.0	11,326
4.41–4.60	203	498.9	11,087
4.61–4.80	364	498.1	10,598
4.81–5.00	552	511.3	10,435
5.01–5.20	641	514.0	10,078
5.21–5.40	625	530.6	10,011
5.41–5.60	648	528.9	9,616
5.61–5.80	511	539.1	9,458
5.81–6.00	359	548.1	9,290
6.01–6.20	236	558.9	9.163
6.21–6.40	128	559.5	8,881
6.41–6.60	79	545.6	8,394
6.61–7.50	65	580.3	8,480

TABLE 6

Analysis of R. of M. records above 700 pounds of butter fat

YEARLY PRODUCTION OF FAT	NUMBER OF COWS	AVERAGE BUTTERFAT PERCENTAGE
pounds		*per cent*
950–1141	39	5.835
900–950	47	5.633
850–900	87	5.637
800–850	161	5.632
750–800	280	5.631
700–750	589	5.487
700–1141	1,203	5.562

TABLE 7

NUMBER OF ANIMALS		MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY	CORRELATION BETWEEN MILK AND FAT PERCENTAGE	CORRELATION BETWEEN TOTAL FAT AND FAT PER CENT
393	Milk..	11433±76.8	2255.0±54.2	19.72±0.47	−0.311±0.0310	
	Per cent fat	5.430±0.018	0.552±0.011	10.16±0.24		
	Total fat	618.8±3.46	101.5±2.44	10.45±0.39		+0.233±0.0324

between milk and fat percentage and between total fat production and fat percentage of these tests.

The results shown by tables 5, 6 and 7 indicate that there is a correlation between total fat production and percentage of butterfat and that the fat production increases as the fat percentage increases. The milk yields shown in tables 5 and 7 agree with the findings of Roberts (1) in that they indicate a negative correlation between fat percentage and milk yields. It is very significant, however, that although this negative correlation exists, nevertheless the milk yields do not decrease in the same ratio as the fat percentages increase. In other words the decline in milk flow is not sufficient to prevent the total fat from increasing as the test increases. This is further confirmed in table 5. If the milk flow declined in proportion as the butterfat percentage increases, there would be no justification for the breeding of higher testing cows.

SUMMARY

1. Butterfat is a variable factor although the degree of variability is less than for milk yield.

2. Both the sire and dam contribute to the inheritance of their daughters, governing fat percentage.

3. A parent may be prepotent in increasing the fat percentage of the offspring separately fróm affecting the milk yield, or may increase or decrease both the percentage of fat and the milk yield.

4. Although there is a negative correlation between milk yield and fat percentage, there is a positive correlation between total fat production and fat percentage.

5. Improvement in total butterfat production can be accomplished by selection for high fat percentage as well as selection for large milk yields.

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SOME OBSERVATIONS ON THE FREEZING POINT OF CREAM AND ITS USE IN DETECTING ADDED WATER*

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The freezing point of milk is a subject that has received a great deal of attention from investigators in widely scattered fields. At present the value is generally accepted as a physiological constant averaging −0.550°C., with a normal variation of from −0.534°C. to −0.562°C. according to Hortvet (1), and from −0.530°C. to −0.566°C. according to Bailey and others (2). The freezing point is now used as a means of detecting added water in milk and a carefully standardized technique has been incorporated, in the methods sponsored by the Association of Official Agricultural Chemists, as an "official method" (3).

The freezing point of cream has not been studied to any great extent. In fact the author has been unable to find any published data dealing with cream as such. However, from cryoscopic data on related substances and a knowledge of the theory of freezing point depression it is not impossible to predict what might be expected.

W. R. G. Atkins (4) found that the addition or removal of fat from milk has no effect on the freezing point.

Parfitt and Taylor (5) show that variations in the fat content of ice cream mixes do not alter the temperature at which they freeze.

Colloidal dispersions, even comparatively concentrated, exhibit very small osmotic pressures and consequently practically negligible depressions of the freezing points of the continuous

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† Published by permission of the Director of the Agricultural Experiment Station. Contribution from the Department of Dairy Husbandry. The Pennsylvania State College, No. 415.

phases, (6) (7). The fat in milk or cream existing as a coarse emulsion, consequently, would not be expected to influence the freezing point of the plasma.

If the fat be removed from milk, skimmilk and cream, either by extraction or centrifugation, the remaining plasmas are identical. Exception to this statement might be taken since Clayton (8) gives data showing that 2 per cent of the casein in ordinary milk is adsorbed or concentrated on the fat globule-plasma interface. Milk in creaming, therefore, might be expected to lose a portion of its colloids to the cream, in which case the respective plasmas might differ. This point, however, would not noticeably affect the freezing point since the colloids, as a class, do not show a noticeable depression phenomenon. The freezing points of milk, skimmilk and cream, then, must be dependent on the concentration of soluble substances in the serum and it can hardly be argued that the serums in question would differ. All of which leads to the conclusion that cream and skimmilk have the same freezing point as the original whole milk from which they are derived.

FREEZING POINTS OF MILK, SKIMMILK AND CREAM

Twelve samples of authentic herd milk were separated and observations made on the resultant skimmilk and cream which were compared with similar observations made on the original whole milk. Cryoscopic readings were obtained following the exact procedure as given in the A.O.A.C. "Official Methods." Fat tests and acidity tests were made in accordance with methods obtained from the same source, with the exception that acidity samples of cream were weighed out instead of measured. The percentages of solids-not-fat represent the difference between the total solids content, obtained by means of the Mojonnier test, and the fat tests.

Elaborate precautions were taken in obtaining the various samples and in holding them prior to analysis and the determination of the freezing points in order that no uncontrolled factors would make a direct comparison impossible. Table 1 gives the data taken on these samples and is chiefly of interest from the standpoint of the cryoscopic results. Samples No. 11 and No. 12 were diluted before separation with 11.5 per cent and 20 per cent of water respectively. The data seems to confirm the theoretical conclusion made above, namely that the freezing point of skimmilk and cream are identical with that of the original whole milk irrespective of their compositions. An elevation of the freezing point due to added water in milk is equally detectable in either the skimmilk or the cream after separation. None of the results on a given sample vary to a greater extent than the possible experimental error. It might be mentioned that the freezing points represent the average of three trials all of which checked within 0.003°C. Results varying outside this range were discarded and a new trial made. The cyroscopic method as given seemed to be equally applicable to cream of a richness and viscosity similar to the samples used. The only difference noted in making cream readings was the relative sluggishness of the mercury thread as compared to the movements with milk.

TABLE 1

Freezing points of milk, cream and skimmilk

SAMPLE NUMBER	WHOLE MILK				CREAM				SKIMMILK			
	Fat	SNF	Acid	Freezing point	Fat	SNF	Acid	Freezing point	Fat	SNF	Acid	Freezing point
	per cent	*per cent*	*per cent*	°C.	*per cent*	*per cent*	*per cent*	°C	*per cent*	*per cent*	*per cent*	°C.
1				−0.558				−0 559				−0.558
2				−0.537				−0.540				−0.538
3				−0.546				−0.546				−0.548
4				−0 544				−0 545				−0.545
5				−0 549				−0.550				−0.548
6	4.30	8.47	0.146	−0.545	22 0	6 85	0 121	−0.545	0.03	8.91	0.157	−0 546
7	3.95	8 51	0.162	−0 538	19 5	7 91	0.156	−0.539	0.04	9.05	0.165	−0.536
8	4 05	8 53	0 145	−0.540	27.5	6 32	0.115	−0 538	0 04	9.02	0.154	−0.537
9	4 00	8.53	0 156	−0 543	24 0	7.02	0 121	−0 545	0 03	9.41	0.161	−0 542
10	4.05	8.36	0.141	−0.541	20 0	7 25	0.118	−0 540	0.03	8 80	0.148	−0.540
11	3.80	7.76	0 135	−0 462	25.0	5.97	0.106	−0.461	0 05	8.17	0 138	−0.460
12	3 35	6.89	0 127	−0 429	21 0	5.92	0 085	−0.430	0 02	7 22	0.135	−0.429

TABLE 2

Watered samples of cream

Freezing points °C.

SAMPLE	UNDILUTED	10 PER CENT ADDED WATER	15 PER CENT ADDED WATER	20 PER CENT ADDED WATER	25 PER CENT ADDED WATER	FAT IN CREAM BEFORE DILUTION
6	−0.545	−0.465	−0.439	−0.386	−0.355	22.0
7	−0.539	−0.464	−0.436	−0.386	−0.357	19.5
8	−0.538			−0.384		27.5
9	−0.545	−0.461	−0.417	−0.386	−0.363	24.0
10	−0.540	−0.465	−0.414	−0.379	−0.353	20.0

Added water calculated from above depressions

$$W = \frac{100\,(T - T')}{T}$$

6	0	14.7	19.4	29.1	34.8	22.0
7	0	13.9	19.1	28.4	33.8	19.5
8	0			28.6		27.5
9	0	15.4	23.5	29.1	33.4	24.0
10	0	13.8	23.3	29.9	34.6	20.0
Average. ...	0	14.4	21.4	29.0	34.1	22.6

Added water calculated from above depressions

$$W = \frac{A\,(T - T')}{T}$$

6	0	10.8	14.7	22.4	27.3	22.0
7	0	10.4	14.8	22.1	26.8	19.5
8	0			21.4		27.5
9	0	11.0	16.9	21.4	25.0	24.0
10	0	10.4	17.7	23.3	27.4	20.0
Average .. .	0	10.6	16.0	22.1	26.6	22.6

DILUTED SAMPLES OF CREAM

Winter's formula given in the "official methods" of the A.O.A.C. for calculating the amount of added water in a sample of milk is as follows:

$$W = \frac{100\,(T - T')}{T}.$$

W, is the percentage of added water; *T*, the average freezing point depression of normal milk (0.550°C.) or the original freezing point depression, where this is known; and *T′*, the freezing point depression of the sample in question. This formula was used in calculating the added water in a series of cream samples which were diluted at the rate of 10, 15, 20 and 25 per cent respectively. These samples are the same as numbers 6 to 10 in Table 1. The freezing points of these samples are listed in the first portion of Table 2, while results obtained using Winter's formula are given in the second portion of the table. It will be noted that the values obtained here are higher than the actual amount of dilution. This is not surprising when the formula is considered in the light of the theoretical conceptions previously mentioned. Winter's formula is accurate only when the volume occupied by the substances in solution is the same as the total volume of the material under observation. Actually then this formula really gives the percentage of added water based on the serum of the sample. Thus, the greater the difference in volume between the serum and the material as a whole, the more inaccurate will the results be when this forumla is used. In samples of whole milk or skimmilk the discrepancy is not so large that results are very far from accurate, especially where the average normal value 0.055°C. is used for the value of *T*, but in cream the differences are considerable as indicated in the table. In using Winter's formula for these results *T* was taken as the freezing point depression of the original undiluted sample.

In the lower portion of the table is shown calculated amounts of water for the samples as in the middle portion, with the exception that a modified formula correcting for the differences in volume between the serum and the cream was used. The formula is as follows:

$$W = \frac{A(T - T')}{T}.^{1}$$

A, is the per cent of serum (by volume) in the sample having a freezing point depression of *T′*. For all practical purposes

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[1] The Cryoscopic method for cream has been accepted tentatively by the A. O. A. C. and this formula is to be used.

the value for A may be taken as the per cent of water in the sample. This is not exactly correct due to a disregarding of specific gravity but is so nearly so that the difference is negligible, especially where the value of T has to be taken as 0.550°C. In this work each diluted sample was not analyzed for water to determine the value of A but the figure was calculated from the analysis of the sample before dilution according to the following formula:

$$A = \frac{b(100 - C)}{100} + C.$$

Here b, represents the per cent of water in the undiluted sample and C, the per cent dilution.

It is noted that the results calculated by the "serum" formula are considerably closer to the actual amounts of water added than the results obtained using Winter's formula. The average algebraic variation in the former is but 1.4 per cent, while in the latter it amounts to 7.3 per cent, roughly five times greater. It is probably unnecessary to state that, when unknown samples of cream are examined, it is necessary to get the value of A by analysis, as it would be rather difficult to calculate it from the percentage of fat when the amount of possible dilution would be unknown.

CREAM SAMPLES FROM DILUTED MILK

Sample No. 11 and No. 12 in table 1 were diluted before separation. Sample No. 11 showed a freezing point depression of 0.536°C., before dilution and sample no. 12 one of 0.558°C. undiluted. Therefore, taking table 1 as evidence the respective cream samples would have had like values in the undiluted state. If the "serum" formula is used and the amount of added water calculated for the cream samples, and in the same way for the skimmilk samples, the results are as follows:

Cream sample no. 11	9.4 per cent added water
Cream sample no. 12	16.8 per cent added water
Skimmilk sample no. 11	13.0 per cent added water
Skimmilk sample no. 12	21.5 per cent added water

Actually there was added to the whole milk samples prior to separation 11.5 per cent and 20 per cent of water respectively. These values are closely approached when calculated from the data by the "serum" formula being 12.2 per cent and 20.7 per cent respectively. It is to be noted, however, that the amounts of added water calculated for the cream and skimmilk portions of these samples do not agree with the amount added to the whole milk, nor do they agree with each other. This of course, is due to the fact that the amounts of serum in the cream and in the skimmilk differ from the amount in the original whole milk and differ more widely as the richness of the cream increases.

In order to determine the amount of water added to a lot of milk prior to separation, from an examination of the cream or the skimmilk, the volumes of each and the amount of added water in each must be known, in which case the following formula may be used.

$$\frac{(C \times W') + (S \times W'')}{C + S} = W.$$

C, is the volume of cream; W' the per cent of added water in the cream; S, the volume of skimmilk; W'', the per cent of added water in the skimmilk and W, the per cent of added water in the original whole milk. This formula naturally would have its limitations when commercial samples are being investigated.

SUMMARY AND CONCLUSIONS

A very brief review of literature, only, is included since no published data was found bearing directly on the subject of this paper.

From data presented it is concluded that cream and skimmilk have the same freezing points as the original sample of milk from which they are separated. If this is so then the normal range must be from −0.530°C. to −0.566°C. as for milk, with an average of −0.550°C.

Winter's formula as given in the "official methods" of the A.O.A.C. is theoretically incorrect and while this fact does not make it entirely inapplicable for whole milk or skimmilk it shows a great degree of inaccuracy when applied to samples of cream, the degree increasing as the cream samples become richer in fat. Data is given showing the inaccuracies when it is applied to a series of diluted cream samples.

A modified formula is given which is theoretically more accurate and when applied to the same samples of cream mentioned above gives fairly close results as compared with actual amounts of water known to have been added.

The amount of added water found in a cream sample or a skimmilk sample does not correspond with the amount added to the original whole milk, in case such diultion was made before separation; but the latter can be calculated by the formula shown if the weights or volumes of both the cream and skimmilk portions are known together with the respective amounts of water in each, as found by cryoscopic examination and the use of the "serum" formula.

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EFFECT OF TEMPERATURE ON THE VISCOSITY OF SKIMMILK*

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INTRODUCTION

Apparently Soxhlet (1) was the first to study the change in viscosity of milk with temperature. He found that the viscosity of whole milk increased faster than water with lowering temperature. His data are not entirely consistent, but Kobler (2), Taylor (3), and Evenson and Ferris (4) confirmed Soxhlet's finding. Soxhlet attributed this change in relative viscosity of milk with temperature to the casein.

The effect of pasteurization on the viscosity of milk was investigated by Woll (5), using the viscometer designed by Babcock (6). Woll pasteurized his samples by heating for twenty minutes at 67°C., and also by heating in an Arnold steam sterilizer for thirty to thirty-five minutes. The samples were heated in Erlenmeyer flasks closed with cotton plugs. He found that pasteurization under these conditions decreased the viscosity of both milk and cream. The decrease in viscosity of milk was about 4 per cent, while the decrease in the viscosity of cream was about 16 to 17 per cent. Woll found that the viscosity of sweet whey was decidedly increased by pasteurization, the increase being still greater after sterilization. Thus the viscosity of whey was increased by pasteurization, while the viscosity of milk and cream was decreased by it.

Babcock and Russell (7) found that pasteurization, as they carried it out, broke up the clumps or clusters of fat globules which are usually present in raw, normal milk, and they attributed the decrease in viscosity produced by pasteurization to breaking up of these clumps of fat globules. Babcock and Russell (8) (9) found that the addition of calcium sucrate restored the

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viscosity of pasteurized cream and also caused clumping. Babcock and Russell (9) also found that the viscosity of pasteurized cream could be restored by incipient churning, but that in this case the fat globules, instead of being characteristic clusters, were lumps, indicating that the fat globules had coalesced. It was found that this cream would not whip.

The importance of clusters of fat globules as affecting the viscosity of milk has been indicated by Babcock (10) in the following words.

> Although these clumps are not very firmly bound together, they have sufficient stability to remain intact under ordinary conditions, and, on account of the increased resistance which these irregular masses offer to a free movement of the serum, add very considerably to the consistency of milk. This is clearly shown in the comparative thinness of pasteurized product, especially cream, in which the grouping is entirely destroyed, leaving the globules uniformly distributed. Moreover, in such milks, the original consistency is restored to a considerable degree by any process which produces a similar tendency to grouping.

Woll (5) found that the viscosity of the skimmilk also decreased slightly after pasteurization, but he presented rather few data showing this effect.

Sterner (11) concluded that the decrease in viscosity of whole milk by pasteurization was not due to the coagulation of the albumin alone, while Jensen (15) attributes the decrease in viscosity to coagulation of the albumin.

Taylor (3) found that heating milk to the pasteurization temperature of around 65°C., and then cooling the milk to 20°C. and determining the viscosity, decreased the viscosity slightly, while pasteurization at higher temperatures produced a slight increase in viscosity.

Evenson and Ferris (4) heated milk for thirty minutes at various temperatures, and then cooled it to 20°C. and determined the viscosity. They found a decrease in viscosity when the milk was pasteurized at 60° to 65°C. Heating milk to 75° to 80°C. increased the viscosity.

Archard and Stassano (12) found that pasteurization failed to effect the viscosity appreciably.

Since most of this work on the effect of heat on the viscosity has been carried out with whole milk, and as Babcock and Russell indicated that the clumping of the fat globules was an important factor in regulating the viscosity of whole milk and cream, and since clumping is usually more pronounced at lower temperatures, it was thought disirable to make a more careful study of the effect of temperature on the viscosity of skimmilk.

EXPERIMENTAL

The viscosity of skimmilk at various temperatures

All determinations were made with ordinary Ostwald viscometers. The temperature was held constant by immersing the viscometers in a water bath held at the desired temperature. The samples were held in the water bath for fifteen minutes before making the readings. The determinations were made on the same sample of milk beginning at the lowest temperature. A duplicate determination with an aliquot, which had been kept cold during the experiment, was made at the highest temperature and was found to agree in viscosity with the value obtained with the sample used for the entire temperature series. The time of flow of water determined experimentally at the various temperatures was used to calculate the constants of the viscometers. The weight of pycnometers filled with milk and then with water was determined at the various temperatures, and from this data the density of the milk was calculated.

The following equation was used to calculate the viscosity

$$\eta = d\left(At - \frac{B}{t}\right)$$

where η is the viscosity in centipoise, d is the density of the milk, t is the time of flow in seconds, and A and B are constants. The constants A and B were calculated from the time of flow of water at the various temperatures and the viscosity of water as given by Bingham and Jackson (13). This equation reproduced the viscosity of water with an average error of less than 0.3 per cent. Three different Ostwald viscometers were used in this work and four samples of mixed skimmilk, two viscometers being used with each sample. The data were plotted and a smooth curve was drawn which most nearly fitted the data.

TABLE 1

The relative and absolute viscosity, density, and specific gravity of skimmilk and a 5 per cent lactose solution at various temperatures

TEMPERATURE	VISCOSITY IN CENTIPOISE			VISCOSITY RELATIVE TO WATER		DENSITY			SPECIFIC GRAVITY		
	Water*	Skimmilk	5 per cent lactose solution	Skimmilk	5 per cent lactose solution	Skimmilk		5 per cent lactose solution	Skimmilk		5 per cent lactose solution
						Experiment 1	Experiment 2		Experiment 1	Experiment 2	
°C. (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
5	1.519	2.96	1.76	1.95	1.16	1.0369	1.0362	1.0204	1.0369	1.0362	1.0204
10	1.308	2.47	1.50	1.89	1.15	1.0360	1.0358	1.0200	1.0363	1.0361	1.0202
15	1.140	2.10		1.84		1.0348	1.0348		1.0357	1.0358	
20	1.005	1.79	1.15	1.78	1.14	1.0338	1.0338	1.0182	1.0356	1.0356	1.0200
25	0.894	1.54	1.03	1.72	1.15	1.0323	1.0321	1.0165	1.0353	1.0352	1.0198
30	0.801	1.33	0.91	1.66	1.14	1.0305	1.0307	1.0155	1.0350	1.0352	1.0199
35	0.723	1.17		1.62		1.0287	1.0288		1.0348	1.0350	
40	0.656	1.04	0.74	1.58	1.13	1.0264	1.0268	1.0119	1.0344	1.0348	1.0198
45	0.599	0.93		1.55		1.0245			1.0346		
50	0.549	0.85	0.62	1.54	1.13	1.0223	1.0223	1.0075	1.0346	1.0346	1.0196
55	0.506	0.77		1.53		1.0198			1.0346		
60	0.469	0.71	0.52	1.52	1.11	1.0171	1.0172	1.0028	1.0344	1.0345	1.0199
65	0.436	0.66		1.52		1.0145			1.0346		
70	0.406	0.62	0.45	1.52	1.11	1.0117	1.0117	0.9972	1.0347	1.0347	1.0199
75	0.380	0.59		1.54							
80	0.357	0.57	0.39	1.59	1.09	1.0052	1.0055	0.9913	1.0343	1.0347	1.0200

* Bingham and Jackson (13).

The values for the viscosity of milk at 5°C. intervals of temperatures were read from the curve and are given in table 1. The relative viscosity was obtained by dividing the true viscosity of milk by the viscosity of water. Thus the term relative viscosity is used in its true sense and not as relative times of flow as is sometimes the case.

The viscosity and density of skimmilk at various temperatures are shown graphically in figure 1. The viscosity of skimmilk decreases as the temperature is raised. The relative viscosity indicates that the viscosity of skimmilk decreases more rapidly than the viscosity of water up to 60°C. Between 60° and 70°C. the viscosity of skimmilk and water change with temperature at the same rate, and above 70°C. the relative viscosity begins to rise again, indicating that the decrease in viscosity of skimmilk as the temperature is raised above 70°C. is not as rapid as the decrease in the viscosity of water. This break in the relative viscosity curve occurs at about the temperature at which heat denaturizes the albumin in milk. This point will be discussed later under the effect of pasteurization on viscosity.

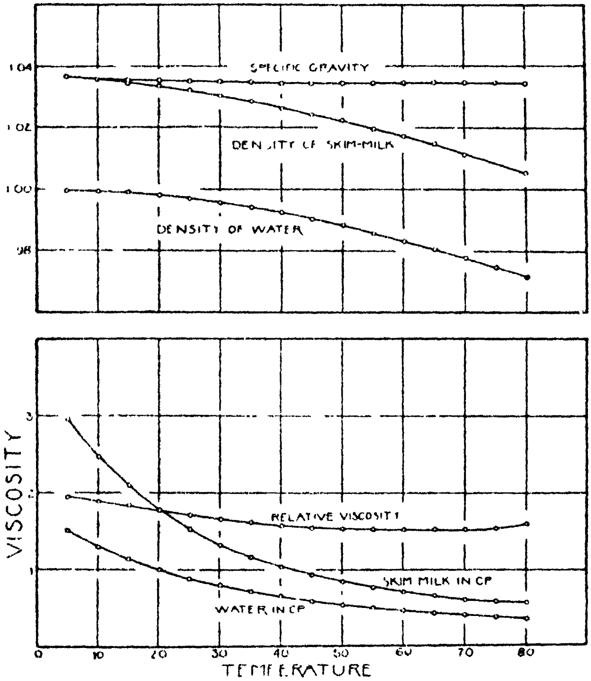

FIG. 1. THE DENSITY, SPECIFIC GRAVITY, ABSOLUTE VISCOSITY, AND RELATIVE VISCOSITY OF SKIMMILK AND WATER AT VARIOUS TEMPERATURES

The relative viscosity of skimmilk was found to be 1.95 at 5°C. and 1.52 at 60°C., or a change in relative viscosity of 0.43. Since the proteins and lactose are the main constituents of skimmilk, the question arises as to whether or not lactose would cause a change in the relative viscosity of milk as the temperature is raised. The viscosity of a 5 per cent solution of lactose at various temperatures is given in column 4, table 1, and the relative viscosity in column 6. The difference in relative viscosity between 5° and 60°C. is 0.04. This experiment indicates that the change in relative viscosity of skimmilk with temperature is due partially to the lactose, but that the effect of the lactose is small in comparison with the effect of the proteins.

It is probable, however, that the effect of both the lactose and the proteins on the relative viscosity is greater when taken together in the same solution than would be indicated by the sum of their individual effects when present alone in solution.

An examination of the density data given in table 1 shows that the density of the skimmilk also decreases more rapidly as the temperature is raised than does the density of water, this difference in rate of change practically all taking place below 40°C. Bowen's (14) data show a similar trend. The density data given in table 1, column 7, were obtained by starting at 5°C. and increasing the temperature in steps up to 80°C. It was feared that air might have been driven out of solution by the heat, and might have remained in the form of fine bubbles dispersed in the milk, which would account for the change. That this was not the case was shown by placing a sample of milk under the vacuum at 35° to 40°C. to remove the air, and then determining the densities in the reverse order starting with the highest temperature. The values obtained by this method are given in column 8, table 1. The total solids content of the skimmilk in experiment 2 was 8.96 per cent, the fat 0.07 per cent, and the proteins 3.22 per cent. The density data were changed to specific gravity, those obtained by increasing temperature are given in column 10; those by decreasing temperature in column 11. The specific gravity data indicate a change in hydration of the milk solids with temperature. This change in hydration seems to be more marked as the temperature of the milk decreases from 40°C. The specific gravity of the skimmilk at 5°C. is 1.0366 and at 40°C. 1.0346, or a difference due to temperature of 0.0020. The density of a 5 per cent solution of lactose is given in column 9, table 1, and its specific gravity in column 12. These data indicate that the specific gravity of a 5 per cent solution of lactose changes 0.0005 between 5° and 40°C.

TABLE 2

Effect on the viscosity determined at 24°C. of pasteurizing skimmilk and rennet whey for thirty minutes at various temperatures

TEMPERATURE OF PASTEURIZATION	SKIMMILK		WHEY	
	Viscosity	Number of samples	Viscosity	Number of samples
°C.	cp		cp.	
Unheated	1.472	62	1.158	9
50	1.434	9	1.146	2
60			1.159	2
62.5	1.440	13		
70	1.455	5	1.176	2
72.5	1.475	4	1.179	2
75	1.490	5		
77			1.193	3
78	1.515	3		
79	1.524	3		
80	1.527	8	1.208	2
82	1.540	3		
85	1.548	3		
90	1.553	4	1.294	2
100	1.603	4	1.381	3
110	1.647	3	1.296	3
120	1.717	3	1.273	4

Thus the lactose of the skimmilk is partly responsible for the change in specific gravity with temperature. Through the same range in temperature a 5 per cent sucrose solution changes 0.0006, which is about the same change as is produced by the lactose, while a 10 per cent sucrose solution changes 0.0013. Thus it is probable that the lactose has less effect on the change in specific gravity with temperature than has the protein material present in the skimmilk. The increase in specific gravity as the temperature is lowered is not, however, sufficiently great to account for the distinct rise in the relative viscosity as the temperature is lowered.

The increase in the specific gravity and in the relative viscosity of skimmilk as the temperature is lowered both indicate that some change has taken place in the skimmilk. This change is probably a variation in the hydration of the milk constituents, probably the casein.

The effect of pasteurization on the viscosity of skimmilk and whey

The skimmilk or whey was pasteurized for thirty minutes at various temperatures, care being taken to prevent loss of moisture. It was then cooled at once to 24°C., held at this temperature for thirty minutes, and the viscosity determined with Ostwald viscometers at this temperature. In order to make the data obtained with the different samples of skimmilk comparable, they were converted to the percentage increase on the basis of the viscosity of the unheated sample, and then converted to the basis of the average viscosity for all of the unheated samples used. The data are given in table 2 and figure 2.

According to these data, pasteurization at temperatures between the normal temperature of the milk as drawn and about 72°C. causes a decrease in the viscosity of skimmilk, while pasteurization above this temperature, up to 120°C., causes an increase in viscosity. There is, however, a definite break in the curve, which occurs at about 83°C., the rate of change from this point on being somewhat less rapid.

The effect of pasteurization on the viscosity of rennet whey was also determined. The results are given in table 2 and figure 2. Pasteurization of whey causes a decrease in the viscosity between 40° and 60°C. From that point on as the temperature of pasteurization is raised, the viscosity increases at a slow rate up to about 75°C., and then at a much faster rate up to 100°C., and above this temperature the viscosity decreases again. The breaks in the whey and the skimmilk curves do not correspond. At the point where the skimmilk changes over to a slower rate, 83°C., the viscosity of the whey has just begun to increase at a

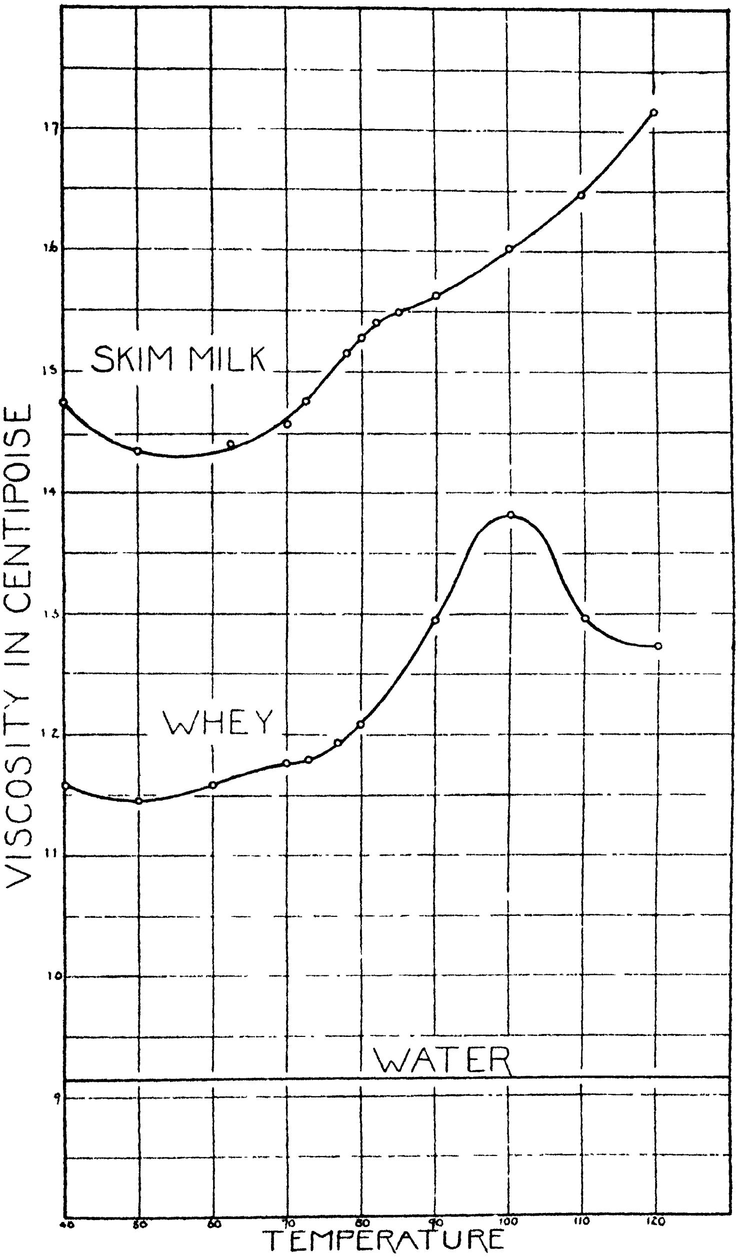

FIG. 2. THE VISCOSITY OF SKIMMILK AND WHEY AT 24°C. AFTER PASTEURIZING FOR 30 MINUTES AT VARIOUS TEMPERATURES

faster rate. The downward break in the whey curve apparently has no counterpart in the skimmilk curve. These results indicate the aggregates formed by heat coagulation of the whey proteins are markedly affected by the presence of the casein.

The data presented here indicate that the decrease in viscosity of milk due to holding pasteurization cannot be due to the coagulation of the albumin for two reasons, first, coagulation of the albumin would tend to increase the viscosity and, second, the maximum decrease in viscosity due to heat occurs at a temperature below the coagulation point of albumin, or where the rate of coagulation would be extremely slow.

The data for the relative viscosity of skimmilk at various temperatures as given in table 1 and figure 1 show a minimum between 60° and 70°C., while the data for the effect of pasteurization on viscosity where the milk is cooled and the viscosity determined at 24°C. indicate a minimum at 50° to 60°C. These minimum ranges are nearly the same as determined by the two methods.

CONCLUSIONS

1. As the temperature is raised from 5° to 60°C., the viscosity of skimmilk decreases faster than the viscosity of water; from 60° to 70°C. the viscosity of both decrease at about the same rate; and above 70°C. the viscosity of skimmilk decreases more slowly than does the viscosity of water.
2. Pasteurization of skimmilk for thirty minutes between 40° and about 72°C. causes a decrease in viscosity, while pasteurization at higher temperatures for the same length of time causes an increase.
3. Pasteurization of whey for thirty minutes at temperatures below 60°C. caused a decrease in viscosity, from 60° to 100°C. an increase, and from 100° to 120°C. a decrease in viscosity.
4. As the temperature is raised from 5° to 40°C. the density of skimmilk decreases faster than does the density of water, while from 40° to 80°C. they both decrease at about the same rate.

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CONCERNING THE ADDITION OF CALCIUM CHLORIDE TO MILK FOR CHEESE MAKING*

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The experiments whose results are given in this report are a continuation of work begun by Georges Knaysi and J. D. Nelson (1). These workers reported an increase in the yield of cheese obtained from a given amount of milk by the addition of $CaCl_2$. They believed that this increase in yield might be due to the retention of more of the milk fat, solids-not-fat, and moisture in the cheese. Their experiments were few in number but pointed out the possibility of producing cheese more economically through the use of $CaCl_2$. They have reviewed the important literature on the subject so that it is necessary here to merely call attention to their report.

The experiments in cheesemaking of the present study have been conducted under carefully controlled conditions which approximate commercial methods of manufacturer accompanied by careful and exact observations and measurements.

The milk used to make the cheese was of varying quality. At least 600 pounds was thoroughly mixed in an ice cream batch mixer after adding starter in amounts sufficient to produce the desired development of acidity during the cheesemaking process. Two portions of exactly 300 pounds each of this mixture of milk and starter were drawn from the mixing tank and placed in two 50-gallon jacketed cheese vats. Each portion was sampled and tested for fat and total solids as a check upon the identity of the milk in each vat.

One-tenth of one per cent or 4.6 ounces of anhydrous $CaCl_2$ was dissolved in 2 pounds of water and added to the test vat of milk. Two pounds of water were added to the check vat to insure the same moisture content in both lots of curd at the time of cutting.

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The $CaCl_2$ stimulated the action of the rennet to such an extent that it was necessary to reduce the amount of rennet added to the milk in the test vat to one-half the amount used in the check vat. Even under these conditions the coagulation of the $CaCl_2$ treated milk was more rapid than the coagulation of the milk in the check vat. Further decreases in the amount of rennet were held to be inadvisable due to the probable effect upon the subsequent ripening of the cheese.

The direct result of the influence of the $CaCl_2$ on the rennet action was a faster and firmer coagulation and a more rapid loss of moisture after cutting. At the time of pressing, however, the moisture contents of the two lots were practically identical. This ability of milk treated with $CaCl_2$ to lose moisture very rapidly during the critical periods of the cheesemaking process has a direct application in the handling of milk with a high acidity at the time of renneting. The shortening of the period in which it is possible to firm the curd sufficiently to insure a sweet cheese allows the cheese maker to remove the whey before the acidity has reached the dangerous limit.

TABLE 1

	AVERAGE YIELD FROM 300 POUNDS OF MILK	POUNDS OF FAT	POUNDS OF SOLIDS-NOT-FAT	POUNDS OF MOISTURE
$CaCl_2$.....	30.388	10.480	9.103	10 800
Check	30.210	10.360	9.015	10.830
Difference	0.178	0 120	0 088	−0 030
Odds that the difference is significant.	132 to 1 Significant	267 to 1 Significant	1.1 to 1 Insignificant	3 4 to 1 Insignificant

The average yield and composition of the cheese obtained from 300 pounds of milk treated with 0.1 per cent of $CaCl_2$ and of the cheese made from 300 pounds of identical milk containing no added $CaCl_2$.

The results of the experiments have been summarized in tables 1 and 2. The average differences in the yield of the cheese and the composition of the cheese and whey have been calculated and the significance of these differences determined by Student's method for interpreting the results of paired experiments (2, 3).

Each 300 pounds of milk treated with $CaCl_2$ produced on the average only 0.178 pound more of cheese than milk not so treated. The real cause of the increase in the yield was obviously the retention of more of the milk fat in the curd. The cheese containing the $CaCl_2$ retained slightly greater amounts of solids-not-fat than the check cheese but the difference was insignificant. The moisture content of the two types of cheese was practically identical.

TABLE 2

	POUNDS OF FAT	POUNDS OF SOLIDS-NOT-FAT
$CaCl_2$	1.017	17.575
Check	1.133	17.502
Difference in pounds	-0.116	0.073
Odds that the difference is significant	9999 to 1 Significant	5 to 1 Insignificant

The average composition of whey obtained from 300 pounds of milk treated with 0.1 per cent of $CaCl_2$ and of the cheese made from 300 pounds of identical milk containing no added $CaCl_2$.

TABLE 3

Average scores

LOT	FLAVOR		BODY AND TEXTURE	
	$CaCl_2$	Check	$CaCl_2$	Check
1	39.7	38.7	22.5	22.8
2	40.0	40.3	23.2	23.2
3	38.8	38.3	22.5	21.7
4	42.0	43.3	22.8	23.8
5	40.3	40.0	23.5	23.5
6	39.0	39.7	22.8	23.2
7	38.7	39.7	22.8	23.0
8	40.3	42.0	23.3	24.2
9	39.3	39.0	23.2	22.8
10	42.2	44.0	23.2	24.0
Mean	40.03	40.50	22.98	23.22

The composition of the whey from the two lots of milk also demonstrates the retention of the fat and other milk solids in the cheese. Table 2 shows that the whey from the $CaCl_2$ treated milk contained 0.116 pound less fat than did the whey from the untreated milk. A slightly greater amount of milk solids not fat in the whey from the $CaCl_2$ treated milk is due probably to chance according to Student's method of interpreting the results of the experiments.

The cheese made in these experiments were scored at eleven months of age. Three judges examined the cheese and their average scores on flavor and body and texture are given in table 3. A score of 50 designates a perfect flavor and 25 a perfect body and texture.

The data of table 3 illustrate the variable quality of the cheese which is due primarily to the quality of the lots of milk from which they were made. Considering the variable nature of the data, the mean scores show little difference in either the flavor or body between the two types of cheese.

The actual scores of the judges were changed to the corresponding ratio scores (4), and these ratio scores were treated by Student's method of determining the significance of the difference of paired observations.

The mean gain in ratio score of the cheese from the untreated over the cheese from the $CaCl_2$ treated milk is 8.18 points. The odds, however, are only 9.4 to 1 that the difference is significant. These odds are too small to state definitely that the $CaCl_2$ milk does not make as good cheese as the non-treated milk.

The mean ratio score of the test cheese is 42.3 ± 3.5 and the cheese from the non-treated milk show an average of 50.4 ± 6.7 points. The small number of observations and the great variation in the quality of the milk from which the cheese were made account for the large probable errors.

The results of the scoring of the cheese do not prove definitely that the $CaCl_2$ has a harmful effect upon the quality of the cheese.

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GRAPHICAL STANDARDIZATION OF CONDENSED MILK PRODUCTS*

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The manufacturer of condensed milk products is confronted with a problem of standardizing peculiar to the condensing industry. The finished products are derived from raw materials whose proper combination has established a desired ratio of fat to milk solids not fat. This ratio is usually stated $\frac{\text{Fat}}{\text{Milk solids not fat}}$. The simple adjustment of these ratios, through the mixing of raw materials like cream, milk, skimmilk, etc., which is rather involved by the usual methods of calculation, can be simplified and shortened by the use of a graph.

For the purpose of explanation it is assumed that an ice cream mix is to be made in the condensing pan which must contain 12 per cent fat, 10 per cent milk solids not fat, 14.5 per cent sugar and 0.5 per cent gelatine, and the completed mix must weigh 3000 pounds. The milk products to be used are milk containing 4 per cent fat and 8.8 per cent milk solids not fat, and cream containing 30 per cent fat and 6.3 per cent milk solids not fat.

It is evident that if it is to be completed in the pan, the mix before condensing should contain the desired amount of the solid elements which should bear to each other the ratio required by the finished product. It is obvious that whatever the combination of milk and cream may be, 435 pounds of sugar and 15 pounds of gelatine must be in the mixture. The information desired, therefore, is the pounds of 30 per cent cream and 4 per cent milk which, combined, will make a mixture containing 12 pounds of fat for each 10 pounds of milk solids not fat, with a total of 360 pounds of fat and 300 pounds of milk solids not fat.

In the graph shown in figure 1 the per cent of fat is plotted on

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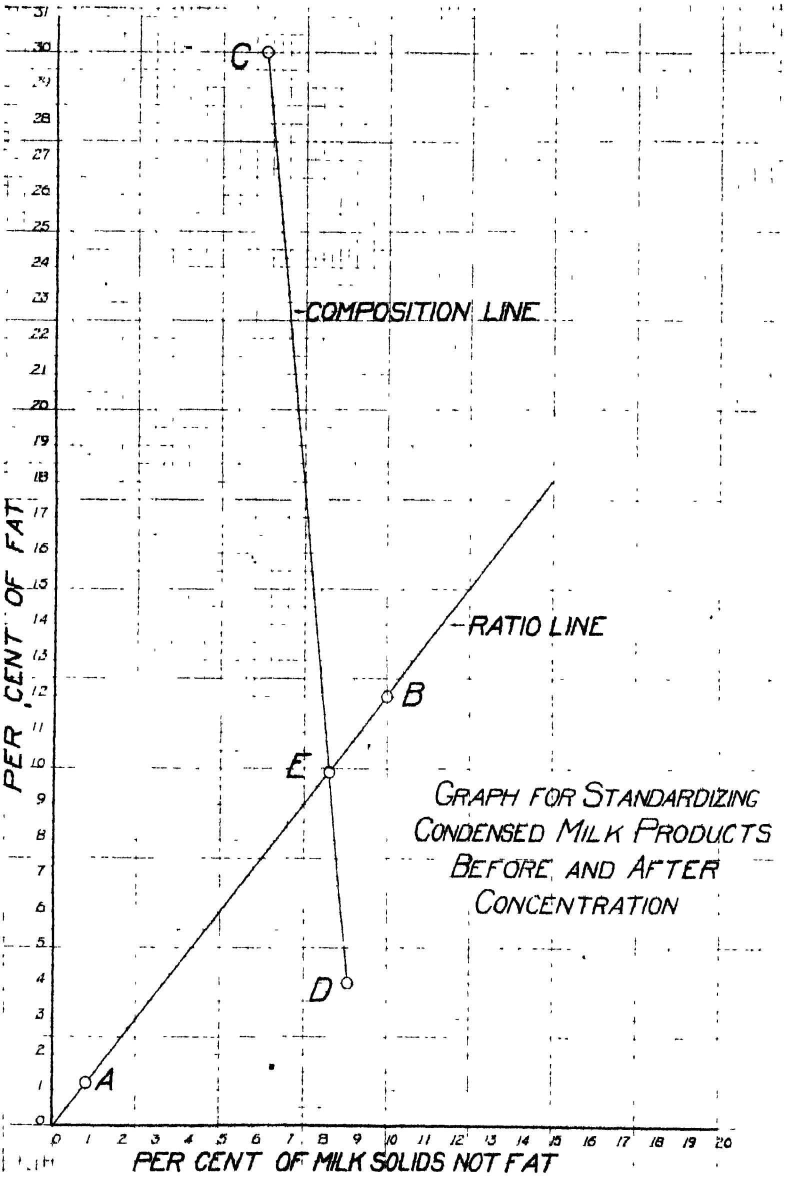


FIG. 1. THE GRAPH ILLUSTRATED WAS DRAWN ON STANDARD, CROSS SECTION, MILLIMETER PAPER TO THE SCALE: 2 CENTIMETERS EQUAL 1 PER CENT

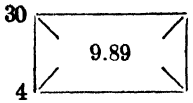
the ordinate and the per cent of milk solids not fat on the abscissa.

The desired mix must contain fat and milk solids not fat in the ratio of $\frac{360}{300}$ or $\frac{12}{10}$ or $\frac{1.2}{1}$, etc. Therefore, if a theoretical mixture of milk products contained 1.2 per cent of milk fat and 1 per cent of milk solids not fat, it would satisfy the ratio of fat to milk solids not fat required by the problem. This composition is represented on the graph by the point *A*. A theoretical mixture of milk products containing 12 per cent of milk fat and 10 per cent of milk solids not fat would also satisfy the ratio of fat to milk solids not fat required by the problem. Point *B* is plotted on the graph to represent this composition. The line determined by the points *A* and *B* is the locus of all points in which the ratio of fat to milk solids not fat is 12 to 10. It will be called "the ratio line" and is determined by the points representing the composition of any two mixtures which contain fat and milk solids not fat in the desired ratio.

A point indicating the composition of the cream is plotted on the graph at *C*. The milk composition is indicated by a point *D*. The line drawn from *C* to *D* contains the points representing the compositions of all the possible combinations of these samples of milk and cream. At *C* the mixture of the two materials contains 100 parts of cream and 0 parts of milk; at *D* the mixture consists of 100 parts of milk and 0 parts of cream. *CD* may be called "the composition line."

The line *CD* intersects the line *AB* at the point *E*, which is the only point in the composition line *CD* which represents fat and milk solids not fat in the ratio of 12 to 10. Point *E* therefore designates the composition of the mixture of milk and cream which when condensed will make a mix containing 12 per cent of fat and 10 per cent of milk solids not fat. Estimated from the position of point *E* on the graph this composition is 9.89 per cent fat and 8.23 per cent milk solids not fat. Several methods can be used to proportion correctly the amounts of milk and cream necessary to make such a mixture.

Pearson's square may be used to determine the pounds of milk and cream necessary to make the mixture of milk and cream containing 9.89 per cent fat and 8.23 per cent msnf. The pounds of this mixture necessary to furnish the total number of pounds of fat and solids not fat can then be calculated. The calculations by the square method may be based upon either the per cent of fat or milk solids not fat in the milk products. It is usually desirable, however, to so adjust the materials that any small error due to incorrect reading of the graph will occur in the amount of milk solids not fat in the total mixture. This will be automatically taken care of if the calculations are based upon the fat composition.



5.89 unit parts of 30 per cent cream

20 11 unit parts of 4 per cent milk

26 00 unit parts of 9.89 per cent mixture

Since 3000 pounds of the mix requires 360 pounds of fat, it follows that the pounds of 9.89 per cent mixture necessary to furnish this amount of fat is $\frac{360}{9.89} \times 100$ or 3640.04 pounds of milk and cream mixture.

Since 5.89 parts of cream and 20.11 parts of milk give 26.00 parts of 9.89 per cent mixture, then the amount of cream required to make 3640.04 pounds of mixture is:

$$\frac{5.89}{26.00} \times 3640.04 = 824\ 61 \text{ pounds}$$

and the amount of milk is:

$$\frac{20.11}{26.00} \times 3640.04 = 2815.43$$

Proof in pounds

	MATERIAL	FAT	MSNF
Milk	2815.4289	112.617	247.757
Cream	824.6088	247.382	51.950
Total	3640.0377	359.999	299.707

No further calculations are necessary. The pounds of sugar and gelatine required may be added to the milk products and the mixture condensed to a total weight of 3000 pounds, when it will have the desired composition.

The proportioning of the amounts of milk and cream can be accomplished in another manner. It has been shown that the line *CD* represents the composition of the mixtures of milk and cream. Since at point *C* there are 0 parts of milk in the mixture, and at point *D* there are 100 parts of milk in the mixture, it follows that points intermediate between *C* and *D* indicate increasing parts of milk per 100 parts of mixture as the positions of these intermediate points approach the composition *D*. From the graph it is evident that point *E* is nearer *D* than *C*, and therefore the desired milk and cream mixture must contain more milk than cream. The exact parts of milk per hundred of mixture would be known if the length of *CE* relative to the length of *CD* were known. It can be shown geometrically that the length of the lines *CE*, *ED*, and *CD* are proportional to the vertical distances between the abscissas in which *C*, *E*, and *D* are located. These abscissas on the graph are designated as 30, 9.89, and 4 per cent respectively. It follows that:

$$\frac{CE}{CD} = \frac{30 - 9.89}{30 - 4} \tag{1}$$

and

$$CE = \frac{(30 - 9.89)\ CD}{30 - 4} \tag{2}$$

The length of the line from *C* to *D* represents an increase of from 0 to 100 parts of milk in 100 parts of mixture, so that *CD* may be said to be equivalent to 100. Equation (2) now becomes:

$$CE = \frac{(30 - 9.89)100}{30 - 4} \tag{3}$$

$$CE = 77.346 \tag{4}$$

The value 77.346 represents the parts of milk in 100 parts of the mixture of milk and cream, and 100 less 77.346 equals 22.654, the parts of cream per 100 parts of milk and cream when the fat content of the milk, cream, and mixture are 4 per cent, 30 per cent and 9.89 per cent respectively.

Since the 3000 pounds of mix required 360 pounds of fat, the pounds of 9.89 per cent mixture necessary to furnish this amount of fat is $\frac{360}{9.89} \times 100$ or 3640.04 pounds of milk and cream. It has been shown that 77.346 per cent of 3640.04 equals the pounds of milk required, and 22.654 per cent of 3640.04 equals the pounds of cream in the mixture. The proof of the method may be shown by making the necessary calculations and arranging the amounts of the products and their fat and milk solids not fat contents in tabular form.

Proof

MATERIAL	POUNDS	POUNDS OF FAT	POUNDS OF MSNF
Milk	2815.42	112.617	247.75
Cream	824.62	247.386	51.95
Total	3640.04	360 003	299.70

No further calculations are necessary. The sugar and gelatine required may be added directly to the milk and cream before or after condensing, as the operator desires. In either case the amount of raw material, which totals 4090 pounds, must be reduced by the removal of water to a total weight of 3000 pounds for the finished product.

The form of graph illustrated has one or two other interesting characteristics and applications. The line *CE* and *CD* might be measured in units of length and the amounts of milk and cream calculated by the proportion

$$\frac{\text{Pounds of milk}}{\text{Pounds of mixture}} = \frac{\text{Length of } CE}{\text{Length of } CD}$$

or

$$\frac{\text{Pounds of cream}}{\text{Pounds of mixture}} = \frac{\text{Length of } ED}{\text{Length of } CD}$$

The graph may be used to standardize off batches of condensed products. No calculations are necessary to determine if the mixture to be standardized is short or over on fat or milk solids not fat. If fat is needed the point representing the composition of the incorrect mixture will fall *below* the ratio line, and if milk solids not fat are needed the point will fall *above* the line. In either case the point representing the composition of the material which can be used to adjust the ratio must fall on the *opposite* side of the ratio line, so that the line connecting the two points will intersect the ratio line. If the point of intersection of the ratio and composition lines indicates a fat and milk solids not fat content in the mixture greater than that desired in the finished product, then water must be added to the milk products. If the point of intersection indicates a fat and milk solids not fat content less than that desired in the finished product, then water must be removed by condensing.

For laboratory work a large graph mounted on a drawing board can be used to good advantage. The ratio lines for the various products manufactured can be drawn on the graph in fine lines and the points representing the composition of the different materials can be marked by glass headed pins which can be inserted where desired and connected by fine black thread in place of drawn lines. This procedure makes it possible to use the graph for a long time without changing the graph paper. A large graph makes possible more accurate standardization.

SUMMARY

1. The ratio line is the locus of all points in which $\frac{\text{Fat}}{\text{Milk solids not fat}}$ equals the ratio desired in the finished product.

2. The line connecting the points representing the compositions of the materials to be combined in the mixture is the locus of all points representing the composition of every possible combination of these materials alone.

3. The intersection of the ratio line and composition line is a point which represents the only combination of the materials used which can furnish fat and solids not fat in the desired ratio.

MOLD AND YEAST COUNTS AND THEIR RELATION TO THE COMPOSITION OF BUTTER*

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The mold and yeast counts of butter have attracted considerable attention during recent years. Lund, Hood, Bouska and others have presented data to show the value of these counts in the control of manufacturing methods. A certain degree of success has been attained in improving the quality of butter through the interpretation of these mold and yeast counts. This improvement has been effected largely through the more efficient pasteurization of the cream and better sanitation in the creamery. Unquestionably, these counts are fair indices of the general sanitation in the creamery. While they cannot be taken per se as criteria of efficient pasteurization, high counts may often be traced to insufficient heating or holding, or both. Routine analyses of butter, including the determination of the mold and yeast counts, make it possible for a supervisory agency to trace difficulties in creamery practice.

Under practical conditions, however, one must always take into account a combination of circumstances, including the time element, temperature and the composition of the butter when the mold and yeast counts are to be interpreted. Bouska points out, for instance, that counts made upon unsalted butter are unreliable after the butter is twenty-four hours old, due to the fact that there is the probability of the growth of the microorganisms particularly if the temperature and other conditions are favorable.

There is a great deal that is still to be learned concerning the factors which influence the development of mold in butter. Thom has demonstrated that the amount of salt present is a

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factor of much importance. However, the information available upon this point is relatively meager and deserves further study. Factors, such as moisture content of the butter, humidity of the atmosphere, temperature, physical and chemical constitution of the butter and others, are challenges for extensive investigation. The utmost value of the mold and yeast counts cannot be reached until we have cleared up some of the unknown variables.

We have under way in our laboratories extensive studies upon the factors influencing the development of mold in butter. In connection with these investigations, we have had occasion to make mold and yeast counts of a large number of samples of butter from several hundred creameries. At the same time, the composition of the butter was ascertained.

The data obtained in this survey serve as a starting point for further studies. The material which is offered at this time is to be considered as preliminary and will be amplified later by controlled experiments.

SOURCE OF MATERIAL

Samples of 2700 lots of butter of varying composition, age and quality were taken at random from 60-pound tubs as they were shipped to the eastern markets.

METHODS OF ANALYSIS

A butter trier was used to remove a plug of butter from the tub. This sample was then placed in a sterile 4-ounce glass jar, provided with an aluminum screw top.

The mold and yeast counts were made after melting the butter at 98° to 100°F. by plating 1 cc. of the melted butter. The plates were poured with whey agar. Before pouring, 1 cc. of 1 per cent sterile tartaric acid solution was added to each plate. The plates were incubated at room temperature (20° to 25°C.) for three days before counting. Counts are reported per cubic centimeter of butter.

The modified Kohman method was used for the determination of the moisture and salt. The curd was arbitrarily assumed as 1 per cent and the fat calculated by difference.

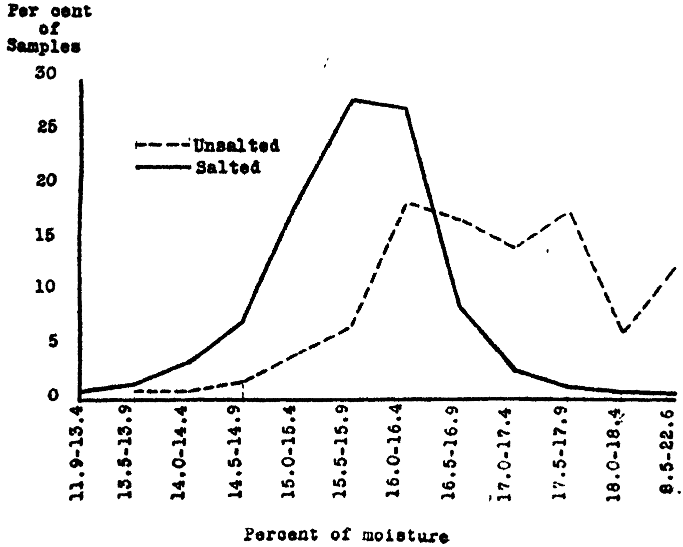

FIG. 1. DISTRIBUTION OF SAMPLES ON THE BASIS OF MOISTURE CONTENT

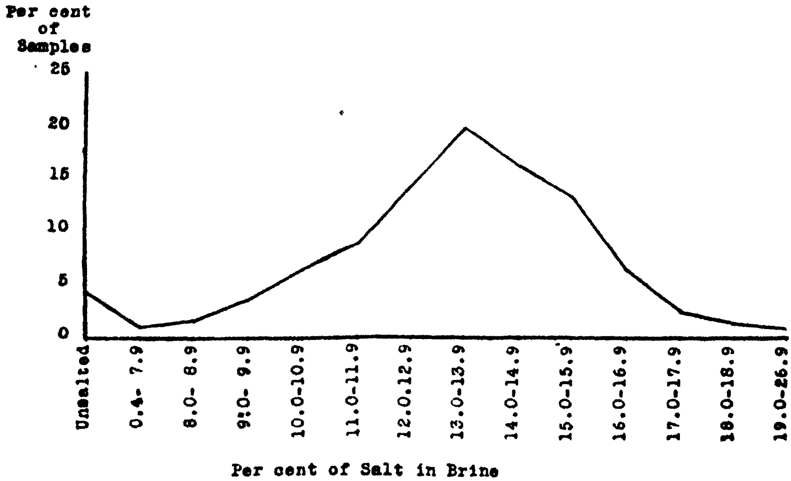

FIG. 2. DISTRIBUTION OF SAMPLES ON THE BASIS OF SALT CONTENT

PRESENTATION OF DATA

The percentage distribution of the samples upon the basis of the percentage of moisture, salt and "salt in brine" is indicated in figures 1 to 3. These serve as a background for the presentation of the tables which follow.

It will be noted that the samples have been arranged in groups in each chart. Some of the extreme groups represent a consolidation of samples over a wide range because of the small number of samples. Otherwise the groups are within limits of 0.5 or 1 per cent, as the case may be. The distribution charts should be self-explanatory.

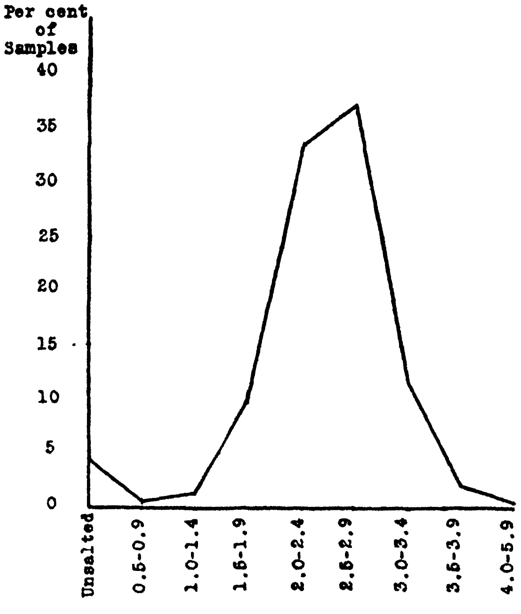

FIG. 3. DISTRIBUTION OF SAMPLES ON THE BASIS OF PERCENTAGE OF SALT IN BRINE

In presenting the mold and yeast counts, individual counts of the samples are not given. It is obvious that it would be impractical to give the figures on each of the 2700 samples. Consequently, the counts on the various samples have been segregated into four classes, namely, those with counts of (*a*) 10 or less, (*b*) 11 to 50, (*c*) 51 to 100 and (*d*) 101 and over.

Counts of 10 or less may be considered as evidence of excellent work in the creamery while those over 100 are to be rated as poor or at best questionable.

The percentage of samples falling into each class has then been determined for each group of samples previously set up on the basis of the composition of the butter. Thus, it will be noted in table 1 that 13.3 per cent of the samples having a moisture content of 11.9 to 13.4 per cent showed a total count of 10 or less, while 33.4 per cent had a total count between 11 and 50, and so on.

TABLE 1

Relation of total mold and yeast counts to moisture content (salted butter)

MOISTURE CONTENT OF BUTTER	DISTRIBUTION OF TOTAL COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
11.9–13.4	13.3	33.4	13.3	40.0
13.5–13.9	10.0	40.0	12.5	37.5
14.0–14.4	9.5	32.6	13.7	44.2
14.5–14.9	11.6	31.8	23.3	33.3
15.0–15.4	15.9	32.2	23.5	28.4
15.5–15.9	13.5	34.4	23.1	29.0
16.0–16.4	18.3	37.1	21.4	23.2
16.5–16.9	10.2	35.4	23.7	30.7
17.0–17.4	15.1	36.4	10.6	37.9
17.5–17.9	3.7	51.9	29.6	14.8
18.0–18.4	13.3	26.7	40.0	20.0
18.5–22.6	8.3	16.7	8.3	66.7

Table 1 illustrates the distribution of the total counts in relation to the moisture content of the salted butter. It will be noted that there is no marked relationship between the count and the moisture content. The increased amount of the moisture in the butter does not seem to reflect any marked increase in the percentage of high counts or vice versa. There is a slight tendency toward higher counts in the high moisture butter but the small number of samples in these extreme groups may temper the significance of this fact.

Due to the limited number of samples of unsalted butter, the figures for them are not reported. They show the same tendency as the salted butters, that is, no marked relationship between total counts and moisture.

TABLE 2

Relation of mold counts to moisture content (salted butter)

MOISTURE CONTENT OF BUTTER	DISTRIBUTION OF MOLD COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
11.9–13.4	53.3	26.7	13.4	6.6
13.5–13.9	45.0	37.5	5.0	12.5
14.0–14.4	47.4	34.7	8.4	9.5
14.5–14.9	47.7	33.3	9.0	10.0
15.0–15.4	51.3	34.0	8.3	6.4
15.5–15.9	48.8	38.3	7.4	5.5
16.0–16.4	54.9	36.1	4.6	4.4
16.5–16.9	46.5	42.3	6.5	4.7
17.0–17.4	43.9	45.5	6.1	4.5
17.5–17.9	55.6	29.6	11.1	3.7
18.0–18.4	46.7	33.3	6.7	13.3
18.5–22.6	25.0	50.0	0.0	25.0

TABLE 3

Relation of yeast counts to moisture content (salted butter)

MOISTURE CONTENT OF BUTTER	DISTRIBUTION OF YEAST COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
11.9–13.4	13.3	46.7	20.0	20.0
13.5–13.9	22.0	36.5	19.5	22.0
14.0–14.4	20.8	36.5	11.4	31.3
14.5–14.9	29.3	35.1	16.8	18.8
15.0–15.4	27.3	41.8	13.3	17.6
15.5–15.9	25.9	40.8	14.7	18.6
16.0–16.4	30.9	39.5	14.6	15.0
16.5–16.9	24.8	36.3	17.1	21.8
17.0–17.4	35.4	23.2	13.4	28.0
17.5–17.9	25.5	29.8	21.3	23.4
18.0–18.4	22.7	36.4	13.6	27.3
18.5–22.6	33.3	8.4	33.3	25.0

In table 2 the data for the mold counts indicate the same lack of direct correlation. This is also true for the unsalted samples.

The relation between the yeast counts and the moisture is shown in table 3. There is no evidence that the moisture content influences the yeast counts. This also holds for the unsalted samples.

TABLE 4

Relation of total mold and yeast counts to salt content

SALT CONTENT OF BUTTER	DISTRIBUTION OF TOTAL COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	0.9	2.7	2.7	93.7
0.5–0.9	0.0	16.7	0.0	83.3
1.0–1.4	6.5	6 5	19.3	67.7
1.5–1.9	10.0	24 4	21.5	44.1
2.0–2.4	12.2	31.9	22 7	33.4
2.5–2.9	17.2	37.1	22.8	22.9
3.0–3.4	16.3	45.7	20 8	17.2
3.5–3.9	22.9	43.8	18.7	14.6
4.0–5.9	42.8	28 6	28.6	0.0

TABLE 5

Relation of mold counts to salt content

SALT CONTENT OF BUTTER	DISTRIBUTION OF MOLD COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	7.0	6.1	8.0	78.9
0.5–0.9	16.7	33.3	0.0	50.0
1.0–1.4	41.9	22.6	16.1	19.4
1.5–1.9	44.1	37.0	7.4	11.5
2.0–2.4	47.2	38.5	8.1	6 2
2.5–2.9	53.4	36.8	5.8	4.0
3.0–3.4	55.3	32.3	7.0	5.4
3.5–3.9	56.3	37.5	2.1	4.1
4.0–5.9	66.7	33.3	0.0	0.0

However, when we come to table 4 we find that there is a marked correlation between the total counts and the salt content of the butter. This is particularly striking in the low and high count columns. As the percentage of salt increases, the percentage of high counts decreases. The intermediate classes do not demonstrate this quite as distinctly as might be expected.

With the mold counts, as shown in table 5 we observe the same tendency for the high counts to be associated with the low salt concentrations. As before, the relationship is particularly marked in the classes with low and high counts.

TABLE 6

Relation of yeast counts to salt content

SALT CONTENT OF BUTTER	DISTRIBUTION OF YEAST COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	28.1	14.0	16.7	41.2
0.5–0.9	50.0	16.7	16.6	16.7
1.0–1.4	25.8	6.5	16.1	51.6
1.5–1.9	21.1	32.9	17.4	28.6
2.0–2.4	21.7	39.5	18.0	20.8
2.5–2.9	31.5	40.3	12.9	15.3
3.0–3.4	34.8	46.6	10.9	7.7
3.5–3.9	43.8	45.8	6.3	4.1
4.0–5.9	42.9	57.1	0.0	0.0

TABLE 7

Percentages of salt in brine with various combinations of salt and moisture in butter

CONTENT OF BUTTER	SALT IN BRINE WHEN PERCENTAGE OF SALT IN BUTTER IS					
	1	2	3	4	5	6
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
20	4.76	9.09	13.04	16.66	30.00	23.08
19	5.00	9.52	13.64	17.39	20.83	24.00
18	5.26	10.00	14.29	18.18	21.74	25.00
17	5.56	10.53	15.00	19.05	22.73	26.09
16	5.88	11.11	15.79	20.00	23.81	27.27
15	6.25	11.77	16.67	21.05	25.00	28.57
14	6.66	12.50	17.65	22.22	26.32	30.00
13	7.14	13.33	18.75	23.53	27.78	31.58
12	7.69	14.28	20.00	25.00	29.41	33.33

The yeast counts do not demonstrate such a sharp curve, as will be noted in table 6. Here there is a general tendency for

TABLE 8

Relation of total mold and yeast counts to salt content of brine

SALT CONTENT OF BRINE	DISTRIBUTION OF TOTAL COUNTS IN EACH GROUP			
	10 or less	11 to 50	50 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	0.9	2.7	2.7	93.7
0.4– 7.9	4.3	4.4	8.7	82.6
8.0– 8.9	8.9	17.8	24.4	48.9
9.0– 9.9	11.4	24.0	19.8	44.8
10.0–10.9	15.3	27.9	18.2	38.6
11.0–11.9	11.1	31.7	25.9	31.3
12.0–12.9	10.3	33.9	21.8	34.0
13.0–13.9	15.1	31.2	24.2	29.5
14.0–14.9	16.1	36.4	23.2	24.3
15.0–15.9	15.7	44.4	20.2	19.7
16.0–16.9	19.6	41.1	22.6	16.7
17.0–17.9	17.6	45.6	23.5	13.3
18.0–18.9	22.2	38.9	19.5	19.4
19.0–26.9	31.8	36.4	27.3	4.5

TABLE 9

Relation of mold counts to salt content of brine

SALT CONTENT OF BRINE	DISTRIBUTION OF MOLD COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	7.0	6.1	8.0	78.9
0.4– 7.9	30.5	21.7	8.7	39.1
8.0– 8.9	42.2	44.4	6.7	6.7
9.0– 9.9	46.9	33.3	10.4	9.4
10.0–10.9	47.2	34.7	6.8	11.3
11.0–11.9	44.0	42.0	8.2	5.8
12.0–12.9	51.3	36.6	5.8	6.3
13.0–13.9	47.7	33.0	7.9	5.4
14.0–14.9	53.4	35.6	6.4	4.6
15.0–15.9	53.9	35.3	6.0	4.8
16.0–16.9	57.7	34.0	3.6	4.7
17.0–17.9	48.5	38.2	5.9	7.4
18.0–18.9	63.9	16.7	16.7	2.7
19.0–26.9	59.1	36.4	0.0	4.5

the high salt percentages to be related with low yeast counts but there are some noticeable irregularities. Yeasts do not appear to be quite as sensitive to salt concentration as the molds.

After these relationships were studied it was thought advisable to investigate the correlation between the concentration of the salt in the moisture and the counts. Consequently, the percentage of "salt in brine" was calculated by dividing the percentage of salt (as determined by analysis) in each sample by the sum of the percentage of salt and moisture.

TABLE 10

Relation of yeast counts to salt content of brine

SALT CONTENT OF BRINE	DISTRIBUTION OF YEAST COUNTS IN EACH GROUP			
	10 or less	11 to 50	51 to 100	101 or more
per cent	*per cent*	*per cent*	*per cent*	*per cent*
Unsalted	28.1	14.0	16.7	41.2
0.4–7.9	34.8	8.7	8.7	47.8
8.0–8.9	24.4	17.8	26.6	31.2
9.0–9.9	19.8	33.3	13.6	33.3
10.0–10.9	27.3	33.5	15.3	23.9
11.0–11.9	22.6	41.2	17.7	18.5
12.0–12.9	18.7	40.0	19.6	21.7
13.0–13.9	26.6	38.7	15.5	19.2
14.0–14.9	30.2	40.4	14.2	15.2
15.0–15.9	33.6	42.5	11.4	12.5
16.0–16.9	33.9	45.3	10.7	10.1
17.0–17.9	41.2	48.5	7.4	2.9
18.0–18.9	47.2	41.7	5.6	5.5
19.0–26.9	36.4	63.6	0.0	0.0

Table 7 is introduced to illustrate how the percentages of salt in brine would vary with different combinations of salt and moisture. This may assist in the interpretation of the remaining tables. It is recognized, of course, that not all of the salt will be uniformly distributed in the water, a fact which will be mentioned later, but the possible relationships of brine and counts are suggestive.

In table 8, the relation between the total counts and the percentage of salt in brine is illustrated. While the effect is not as regular as that of salt, it is quite marked, in the classes of low and high counts and particularly the latter. With the increase in the concentration of salt in brine, there is a corresponding decrease in the number of samples with high counts.

When we consider the mold counts in table 9, we find that the effect of the salt concentration in the brine is again marked, although it is not as distinct an effect as that shown by the salt alone. However, it is significant. Here again high mold counts are correlated with low salt concentration.

As we might predict, table 10 shows that the yeasts are not as sensitive to the salt, although there is a somewhat general trend toward higher counts with low percentages of salt in brine.

DISCUSSION OF RESULTS

These samples of typical market butter, varying in age from about one day to two weeks were analysed without regard for that fact. The chemical analyses paralleled the making of the mold and yeast counts. The results simply indicate the possible relationship between the composition of the butter and the mold and yeast counts. They are at least suggestive. There has been very meager information in the literature to show such relationships, especially on a large number of samples of butter. However, it is clearly understood that the results must be interpreted with judgment. We do not know how much effect the various constituents of butter may actually have had upon the growth of the molds or yeasts from the time the butter was made until it was analysed. At any rate, some very good clues have been uncovered. Investigations to elucidate causal factors are now in progress in our laboratory. The results should throw some light on the subject and clear up some of the puzzling details.

There is also a factor to be considered in the interpretation of mold and yeast counts, such as those reported, namely, individual workmanship in a creamery. The operator who is careless about the control of the composition of the butter may be likewise careless about sanitation and pasteurization. This may have influenced discrepancies in the extreme groups.

The most striking feature of the data presented is to be found in the correlation between the salt content of the butter and the mold and yeast counts. The effect of the concentration of salt in brine appears to be significant.

It is well to bear in mind, however, when we are considering the relationship of moisture, salt or brine, that, while the ordinary butter analysis may show, gravimetrically, the total amount of salt or moisture in the entire sample of butter, it does not reveal the nature of the dispersion, the amount of dissolved or undissolved salt, or whether the particles of salt or water, and the combinations of the two are evenly or unevenly distributed throughout the sample. Many of the secrets of the defects in the keeping quality of butter may be found to be hidden in the physico-chemical makeup of butter and the relative distribution of the particles of water, salt, curd and air. The solution of at least a part of the problem of the factors influencing the growth of mold in butter may be found in these delicate balances.

SUMMARY

1. Data are presented for 2700 samples of market butter, setting forth the mold and yeast counts and the composition.
2. The mold and yeast counts are compared with the concentrations of moisture, salt and "salt in brine."
3. Apparently, the moisture content of the butter had no particular relationship to the counts.
4. The mold and yeast counts appeared to be effected to a marked degree by the salt content of the butter.
5. The percentage of "salt in brine" seemed to show an effect upon the mold and yeast counts.
6. The influence of higher concentrations of salt and "salt in brine," appeared more marked for mold than for yeast counts.

INCREASING THE YIELD OF CHEESE BY THE ADDITION OF CALCIUM CHLORIDE TO MILK*

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As a result of his work on the chemistry of milk, Lindet pointed out, in 1913, the possibility of increasing the yield in cheese, from a given amount of milk, by the addition to the milk of $CaCl_2$. A few trials were made by some French cheesemakers, and an increase varying from 1.50 to 7.22 per cent was reported. The experiments reported, however, were not conducted on a scientific basis, and no attempt was made to determine the source of that increase, which might have been due to an increase in the moisture content of the cheese, to a decrease of the fat losses in the whey, to a more complete precipitation of the other milk solids, or to a combination of some or all of the above factors.

The following experiments are preliminary to more extensive ones which will be carried on in this department in order to obtain more complete and accurate data in regard to the magnitude of that increase, its source, and the effect of $CaCl_2$ on the flavor, character, and ripening qualities of American cheddar cheese.

Table 1 sums up the results of four experiments which have been conducted with American cheddar cheese. The milk was mixed in a vat, and two equal quantities weighed out and subsequently treated alike as much as possible, except that one portion received $CaCl_2$ and a smaller amount of rennet extract, so that both portions coagulated nearly at the same rate. The cheeses were weighed as soon as taken out of the press and sampled, and the samples were tested for fat and total solids.

Table 1 shows that a certain increase in cheese was always secured when $CaCl_2$ was added to the milk previous to the addition of rennet extract. The increase probably varies with the

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TABLE 1

Magnitude and source of the increase in cheese resulting from the addition of $CaCl_2$ to the milk

NUMBER OF EXPERIMENTS	WEIGHT OF MILK	TREATMENT	YIELD OF CHEESE	INCREASE IN YIELD DUE TO $CaCl_2$		COMPOSITION OF THE CHEESE	
						Fat	Total solids
	pounds		*pounds*	*pounds*	*per cent*	*per cent*	*per cent*
I	281.0	3.4 ounces dry $CaCl_2$	31.4	1.5	5.0	32.0	59.52
	281.0	No $CaCl_2$	29.9			32.0	59.4
II	283 0	3.4 ounces dry $CaCl_2$	33.7	1.9	6 0	33.5	63.15
	283.0	No $CaCl_2$	31.8			33.5	63.64
III	282.5	4.4 ounces crystallized $CaCl_2$	28.7	1.0	3.6	32.5	62.64
	282 5	No $CaCl_2$	27.7			31.0	63 02
IV		4.4 ounces crystallized $CaCl_2$	26 06	0.88	3 5	30.5	62.61
		No $CaCl_2$	25.18			31.5	62.90

TABLE 2[*]

Sources of increase in yield of cheese of $CaCl_2$ treated milk

POUNDS OF CHEESE FROM 'HE SAME QUANTITY OF MILK		POUNDS OF FAT IN THE CHEESE		POUNDS OF S.N.F. IN THE CHEESE		POUNDS OF MOISTURE IN THE CHEESE	
$CaCl_2$	None	$CaCl_2$	None	$CaCl_2$	None	$CaCl_2$	None
31.4	29.9	10.048	9 568	8 641	8.193	12.710	12 129
33 7	31.8	11 289	10.653	9.992	9.584	12 418	11.562
28 7	27.7	9 327	8 587	8 650	8.869	10.722	10.243
26.06	25.18	7.948	7 932	8.368	7.906	9.743	9 342

Totals

119.86	114.58	38 612	36 740	35.651	34.552	45 593	43.286

Gain of $CaCl_2$ in yield and content of fat, S.N.F., and moisture of $CaCl_2$ over none

+5.28		+1.872		+1 099		+2.307	

Total gain of each ingredient = 5.278.

Total gain of cheese = 5.28.

[*] We owe this table to the courtesy of Dr. W. V. Price of this department. It is based on the data given in table 1.

composition of the milk, and also with the amount of $CaCl_2$ added. In experiments III and IV, crystallized $CaCl_2$ was added, and probably that was mainly responsible for the drop in the percentage of increase. One-tenth of one per cent of anhydrous $CaCl_2$ should be added.

In table 2 the sources of that increase are shown to be due to more moisture and fat incorporated, and to an increase in the milk-solids-not-fat retained in the cheese. The firmness of the curd from the milk to which $CaCl_2$ has been added might have cut down the amount of small particles carried away in the whey, but other work that we have done shows that there is a more complete precipitation of the soluble milk solids and seems to confirm Lindet's finding that the addition of $CaCl_2$ to milk results in the precipitation of 4.2 to 15.1 per cent of the soluble milk proteins, but we did not check up his other report that at the same time 26.0 to 40.9 per cent of the soluble milk phosphorus is precipitated.

While the data on hand is still scanty, it shows, nevertheless, remarkable economic possibilities. Not only do we get about 5 per cent more cheese from a given amount of milk, but there is a saving of rennet extract, and we see also possibilities in reducing the amount of starter needed. The curd is more easy to handle and the cheese probably has a superior nutritive value due to a more complete precipitation of the phosphorus and the calcium, while there are yet no indications that either the flavor or the ripening qualities of the cheese are impaired.

The chemical changes involved may be very complex, but it seems probable that, due to the condition of dynamic equilibrium in which the soluble salts of the milk serum probably are, the addition of $CaCl_2$ to milk favors the formation of the insoluble calcium phosphates and citrates at the expense of the phosphates and the citrates of sodium and potassium in which the caseins of the milk are soluble, as shown by Lindet. That, and the depressing effect of the $CaCl_2$ upon the dissociation of calcium caseinate with which it has a common ion, probably explain the more complete precipitation which takes place on the addition of $CaCl_2$ to milk.

We feel thus justified in advocating a thorough trial of the use of $CaCl_2$ in cheese making, and we hope that more data will be forthcoming soon in regard to the points touched on in this paper.

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THE NON-PROTEIN NITROGEN IN CERTAIN DAIRY RATIONS AND THE PARTITION OF NITROGEN IN THE URINE PRODUCED THEREON*

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In a recent publication from this laboratory by Maynard, Miller and Krauss (1) there are reported some nitrogen balance studies comparing the protein efficiencies for milk production of a clover ration and a timothy ration. In these studies digestible crude protein was the basis used for computing the experimental rations and also for comparing certain of the results obtained.

It was recognized that the rations being compared contained a large proportion of their nitrogen in compounds other than proteins and that, since in one ration a much larger proportion of the nitrogen was supplied by roughage than was the case in the other, the rations presumably differed markedly in the forms of nitrogen making up what was considered as digestible crude protein. In view of the difference of opinion as to the usefulness of non-protein nitrogen in animal nutrition and the adoption by certain teachers of the Armsby feeding standard which considers non-protein nitrogenous compounds of no value except as a source of energy, it seemed worth while to embrace the opportunity provided by the nitrogen-balance experiment to study the relative amounts of protein and non-protein nitrogen in the rations, and the partition of this ingested nitrogen in the urine and feces. It was believed that such a study would furnish further data on the question as to whether digestible true protein or digestible crude protein is the better basis for computing protein requirements, and that it would also aid in the interpretation of the

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results of the nitrogen-balance experiment. This paper presents the results of an attempt to study these questions.

REVIEW OF LITERATURE

In 1911, Armsby (2) summarized the evidence regarding the nutritive value of non-protein nitrogen from which he concluded that this nitrogen is much inferior to protein nitrogen for production feeding. In 1917, Armsby (3) again discussed the question, stating that digestible true protein seems a safer basis for the computation of rations. On the other hand the reasons why the amids should not be ignored have been stated by Henry and Morrison (4).

In 1924, Forbes (5) reviewed the question of crude and true protein, deciding that the distinction had largely lost its significance in view of the newer knowledge of protein katabolism and that the use of the crude protein standard should be continued. More recently Mitchell (6) has also expressed a similar view.

Actual experiments regarding the replacement value of non-protein for protein had been carried out principally by German investigators. The idea was early advanced that microörganisms in the digestive tract of ruminants may convert non-protein into protein and the early work along this line has been reviewed by Armsby (2). Within the past few years a number of German investigations have been published which it seems worth while to review because of their contribution to the question of crude and true protein.

Völtz (7), using a full-grown wether, found that a ration in which slightly more than half of the nitrogen was furnished by urea allowed 1.2 grams of nitrogen daily for meat production. From previous trials, it was computed that the use of urea had increased the digestible nitrogen 77.7 per cent.

On a ration in which the only source of nitrogen was urea, the same investigator increased the weight of a wether lamb in nine months from 29.29 kgm. to 41.2 kgm., or 40.8 per cent of the initial weight. The explanation given for this is that the urea was built up into bacterial protein in the digestive tract, and 80 to 90 per cent of this bacterial protein was absorbed. It was calculated that 350 grams of urea would replace 1000 grams of protein. Völtz considers as unproved the view that the amids are worthless as a food for ruminants because they are alleged to have no worth in a production ration, and maintains that the nitrogen content of a ration be measured in terms of digestible crude protein rather than digestible true protein. Salkowski (8) objected to

Völtz's interpretation on the ground that the necessary sulfur for protein formation could not have been furnished by the hydrolyzed straw used in the rations.

Morgen and coworkers (9), experimenting with lactating sheep and goats over a period of several years, found that when 30 to 40 per cent of the protein of a ration suitable for milk production was replaced with urea no appreciable change occurred in the milk production. In the following year, Morgen, Windheuser and Ohlmer (10) pointed out that too much urea was unsuitable.

Following up his earlier investigations with an extensive series of studies on dairy cows, Völtz and coworkers (11) were able to furnish additional proof of the replacement value of urea for milk production. As a result of these trials, the authors recommend supplementing protein-poor rations for cattle and sheep with urea, about 150 grams per day being considered the optimum amount.

Similar results were obtained by Honcamp, Koudela and Müller (12) who found that cows will handle from 150 to 200 grams of urea per head per day without ill effects of any kind, and that on a protein-poor, but easily digestible carbohydrate-rich ration a supplement of urea causes an increase in the amount of milk and fat produced.

Morgen, Windheuser and Ohlmer (13) did not obtain an increase in milk production in sheep and goats by adding urea to low protein rations, but did in almost every case obtain an increase in the amount of fat produced.

Further evidence of the truth of the protein-bacteria theory was obtained by Honcamp and Schneller (14), working with wethers, who found that urea, when added to a ration consisting of roughages, was almost quantitatively excreted in the urine. When easily digestible, carbohydrate-rich feeds were given, a marked positive nitrogen balance resulted. On such a ration, in addition to the activity of the bacteria, a protein sparing action is brought about by the combination of ammonia from urea with organic acids from the carbohydrates.

More light is thrown upon the bacterial activity occurring in the first stomach of ruminants by Schwarz (15). In 100 kgm. of paunch contents he found 2.79 kgm. of microörganisms containing 41 grams of nitrogen or 256 grams of protein, 80 to 90 per cent of which, according to Völtz, could be utilized. It was suggested as being possible that a great part of the protein requirement of ruminants is satisfied indirectly by microörganism-protein into which the plant nitrogen is converted in the paunch. It is also possible that specific organisms break down the food while others assimilate it. Medium hay contains about 3 per cent digestible protein whereas the microörganisms in the paunch contained 7.4 to 8.3 per cent digestible protein. The author believes that unquestionably through such a concentration of the digestible protein in the paunch its further usefulness in the intestine is made possible.

Based upon the results of the investigations just cited, it would seem that in ruminants the change of non-protein to protein by bacteria may be a considerable factor, and to the extent that this bacterial synthesis occurs the question of quality of protein may be of little importance in ruminants.

EXPERIMENTAL PROCEDURE

The nitrogen-balance experiment, in connection with which the study here described was made, has been reported by Maynard, Miller and Krauss (1) and the reader is referred to this publication for a detailed account of the experimental procedure which furnished the materials for the study here reported.

The plan for the nitrogen-balance experiment provided that three cows were to receive in successive periods a ration of clover hay, silage and grain, and a ration of timothy hay, silage and grain, each ration to supply equivalent amounts of digestible crude protein and total digestible nutrients in accordance with the weight and production of each cow. It was stipulated that the intake of total digestible nutrients should be adequate according to the Morrison standard, but that the protein intake should be limited to an amount that would throw the animals into negative nitrogen balance, under which conditions a comparison of the relative efficiency of the nitrogen of the two rations would become possible.

The studies described in the present paper were made upon the samples of feeds, feces and urine obtained in the nitrogen-balance studies made in two successive years. The first year's study, comprising trial 1 in which clover hay was used, and trial 2 in which timothy hay was used, was carried out in the winter of 1923–1924. The second year's study, comprising trial 3, timothy, and trial 4, clover, was carried out during the corresponding period of 1924–1925.

The feeds and rations

The ingredients of the grain mixtures and the average daily rations consumed are shown in table 1. In trial 1, 35 grams of salt per day were fed; in trial 2, 45 grams, and in trials 3 and 4, 60 grams.

Substantially the same amounts of hay and silage were used in all trials,[1] the grain being varied in kind and amount to supply the additional digestible crude protein and total digestible nutrients necessary to meet the requirements with the roughages in question. Thus, with clover hay and silage, the grain supplied a much smaller percentage of the protein in the ration than where timothy and silage were used as the roughages.

TABLE 1

Ingredients of grain mixtures and average daily rations consumed

TRIAL	COW	COMPOSITION OF GRAIN MIXTURE				TOTAL GRAIN	TIMOTHY HAY	CLOVER HAY	SILAGE
		Corn meal	Ground oats	Wheat bran	Oil meal				
		per cent	*per cent*	*per cent*	*per cent*	*grams*	*grams*	*grams*	*grams*
1	1	59 67	20.17	13 44	6.72	8,358		5,444	18,144
	2	45.19	27.41	18.27	9.13	6,276		5,226	18,144
	3	54.65	22.67	15.12	7.56	10,134		5,225	17,517
2	1	35 29	32 36	21 57	10.78	8,970	5,444		18,144
	2	25.19	37.40	24 94	12.47	6,794	4,536		12,096
	3	26.79	36.61	24.40	12 20	10,722	5,444		18,050
3	4	33.42	33 29	22.19	11.10	8,934	5,460		18,144
	5	35.22	32.39	21.59	10.80	7,146	5,460		18,144
	6	40.79	29.61	19.73	9 87	7,244	4,527		17,085
4	4	36.89	63.81			8,544		5,460	18,144
	5	61.20	38.80			7,224		4,540	18,144
	6	65.47	34.53			6,438		4,540	16,689

The grain mixture for every trial, except number 4, consisted of corn meal, ground oats, wheat bran and linseed oil meal. In trial 4 it was necessary to limit the grain mixture to the two concentrates lowest in protein to provide the desired plane of protein intake. The clover used in trial 1 was graded on the federal basis as number 2 heavy clover mixed; that used in trial 4 was graded as number 1 clover. The timothy hay used in trial 2 was graded number 2 timothy; that used in trial 3 was graded number 1 timothy. The silage used consisted for the most part of corn which was rather immature and contained few ears.

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[1] The exceptions to this statement noted in the table are explained in the publication by Maynard, Miller and Krauss (1).

Distilled water was fed during trials 1 and 2, but during trials 3 and 4 tap water was given. From the results of a recent analysis carried out by Dr. F. R. Georgia of the Department of Chemistry, of the water supply used in trials 3 and 4, it was found that the amount of nitrogen in the water was negligible.

Chemical methods[2]

The feeds and feces were analyzed for total and non-protein nitrogen. The Kjeldahl-Gunning method was used for the total nitrogen determination. Non-protein nitrogen was obtained by analyzing for true protein nitrogen by the albuminoid nitrogen method adopted by the Association of Official Agricultural Chemists and subtracting this result from the total nitrogen.

It was recognized, in view of the recent work on the distribution of nitrogen in proteins and in plant tissues, that this method might have limitations as an accurate measure of the non-protein nitrogen. However, it is the method that has been employed wherever distinctions have been drawn between crude and true protein, and it is upon data obtained by this method that the protein requirement of the Armsby and Kellner feeding standards are based, on the usefulness of which the present studies were expected to throw some light. Both because of these facts and because no other generally recognized method of greater value was available, the method here used was chosen.

For trials 1 and 2, urea nitrogen was determined by the Folin and Youngburg method (16) after extracting the ammonia with permutit. No analysis for ammonia nitrogen was made on the urine collected in trials 1 and 2. In trials 3 and 4 special urine samples were acidified with sulphuric acid for the ammonia determination. Upon analyzing these acidified samples for ammonia by Folin and Bell's permutit method (17) no appreciable amount of ammonia could be detected. Likewise, when analyzed for urea by Folin and Youngburg's method (16) and by the new Sumner method, no appreciable amount of ammonia was found. Thinking that the acidity might be the cause of this, the same methods for determining ammonia and urea were applied to unacidified samples of the same urine, but similar results were obtained. The aeration method of Folin for ammonia as described by Hawk (18) and the aeration method of Van Slyke and Cullen (19) for urea were then tried and reasonable amounts of ammonia and urea were found. The failure of the other methods might have been due to the presence of some compound, possibly one containing an amino group, which prevented the action of Nessler's solution.

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[2] Except for the total nitrogen determination, all urine analyses were made in the biochemistry laboratory of the Cornell Medical College.

Hippuric acid nitrogen was determined throughout by the method of Folin as modified by Kingsbury and Swanson (20).

Creatinine and creatine nitrogen were not determined in any of the trials reported here. However, in order to help explain the large amount of undetermined nitrogen in the urine, these two constituents were analyzed for in the urine of a cow used in a later trial of the nitrogen-balance experiment not included in this paper. Folin's microchemical method (21) was used for both creatinine and creatine.

RESULTS

The distribution of non-protein nitrogen in the feed is given in table 2. It will be seen from a study of this table that the timothy hay used in both cases had a greater percentage of its total nitrogen in the form of non-protein nitrogen than did the clover hay, the figures being, in per cent of total nitrogen, 16.12 and 19.78 for timothy hay, and 2.34 and 10.38 for clover hay. This is in agreement with the results obtained by Grindley and Eckstein (22)[3] who found 15.43 per cent of the total nitrogen of timothy

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[3] Colloidal ferric hydroxide used as precipitant.

TABLE 2

Nitrogen content of feeds and feces

COW	FEED	FED (1)	TOTAL N (2)	TOTAL N (3)	NON-PROTEIN N (4)	NON-PROTEIN N (5)	TOTAL N AS NON-PROTEIN N (6)	TOTAL N IN RATION AS NON-PROTEIN N IN ROUGHAGE (7)	NON-PROTEIN N OF RATION FURNISHED BY EACH FEED (8)	NON-PROTEIN N IN FECES (9)
		Trial 1 (clover)								
		grams	*per cent*	*grams*	*per cent*	*grams*	*per cent*	*per cent*	*per cent*	*grams*
1	Hay	5,444	1.864	101.48	0.043	2.34	2.31		7.01	
	Silage	18,144	0.348	63.14	0.114	20.68	32.76	7.058	61.95	24.56
	Grain	8,358	1.934	161.64	0.124	10.36	6.41		31.04	
	Ration	31,946	1.021	326.26	0.105	33.38	10.23			
2	Hay	5,226	1.864	97.41	0.043	2.25	2.31	7.970	5.57	
	Silage	18,144	0.348	63.14	0.114	20.68	32.76		51.23	11.57
	Grain	6,276	2.026	127.15	0.278	17.45	13.72		43.21	
	Ration	29,646	0.970	287.71	0.136	40.38	14.03			
3	Hay	5,225	1.864	97.39	0.043	2.25	2.31		4.23	
	Silage	17,517	0.348	60.96	0.114	19.97	32.76	6.107	37.59	15.91
	Grain	10,134	2.027	205.42	0.305	30.91	15.05		58.18	
	Ration	32,876	1.106	363.77	0.162	53.13	14.60			
		Trial 2 (timothy)								
1	Hay	5,444	0.825	44.91	0.133	7.24	16.12		13.73	
	Silage	18,144	0.390	70.76	0.141	25.58	36.15	10.391	48.51	21.747
	Grain	8,970	2.232	200.21	0.222	19.91	9.95		37.76	
	Ration	32,558	0.970	315.88	0.162	52.73	16.70			
2	Hay	4,536	0.825	37.42	0.133	6.03	16.12		16.27	
	Silage	12,096	0.390	47.17	0.141	17.06	36.15	9.687	45.99	5.237
	Grain	6,794	2.263	153.75	0.206	14.00	9.10		37.74	
	Ration	23,426	1.017	238.34	0.158	37.09	15.56			
3	Hay	5,444	0.825	44.91	0.133	7.24	16.12		9.27	
	Silage	18,050	0.390	70.40	0.141	25.45	36.15	8.890	32.57	13.001
	Grain	10,722	2.354	252.40	0.424	45.46	18.01		58.17	
	Ration	34,216	1.075	367.71	0.228	78.15	21.25			

TABLE 2—*Continued*

COW	FEED	FED (1)	TOTAL N (2)	TOTAL N (3)	NON-PROTEIN N (4)	NON-PROTEIN N (5)	TOTAL N AS NON-PROTEIN N (6)	TOTAL N IN RATION AS NON-PROTEIN N IN ROUGHAGE (7)	NON-PROTEIN N OF RATION FURNISHED BY EACH FEED (8)	NON-PROTEIN N IN FECES (9)
Trial 3 (timothy)										
		grams	*per cent*	*grams*	*per cent*	*grams*	*per cent*	*per cent*	*per cent*	*grams*
4	Hay	5,460	0.723	39.48	0.143	7.81	19.78		16.01	
	Silage	18,144	0.292	52.98	0.144	26.13	49.31	12.929	53.58	16.52
	Grain	8,934	1.903	170.01	0.166	14.83	8.72		30.41	
	Ration	32,538	0.807	262.47	0.150	48.77	18.58			
5	Hay	5,460	0.723	39.48	0.143	7.81	19.78		15.52	
	Silage	18,144	0.292	52.98	0.144	26.13	49.31	14.413	51.94	8.96
	Grain	7,146	2.001	142.99	0.229	16.36	11.44		32.53	
	Ration	30,750	0.766	235.45	0.164	50.30	21.36			
6	Hay	4,527	0.723	32.73	0.143	6.47	19.78		15.33	
	Silage	17,085	0.292	49.89	0.144	24.60	49.31	13.872	58.25	4.69
	Grain	7,244	1.952	141.40	0.154	11.16	7.89		26.42	
	Ration	28,856	0.776	224.02	0.146	42.23	18.85			
Trial 4 (clover)										
4	Hay	5,460	1.995	108.93	0.207	11.30	10.38		32.21	
	Silage	18,144	0.237	43.00	0.084	15.24	35.44	9.868	43.44	12.92
	Grain	8,544	1.370	117.05	0.100	8.54	7.30		24.35	
	Ration	32,148	0.837	268.98	0.109	35.08	13.05			
5	Hay	4,540	1.995	90.57	0.207	9.40	10.38		20.75	
	Silage	18,144	0.237	43.00	0.084	15.24	35.44	10.188	33.65	14.00
	Grain	7,224	1.499	108.29	0.286	20.66	19.08		45.61	
	Ration	29,908	0.809	241.86	0.151	45.30	18.73			
6	Hay	4,540	1.995	90.57	0.207	9.40	10.38		26.32	
	Silage	16,689	0.237	39.55	0.084	14.02	35.44	10.604	39.25	14.70
	Grain	6,438	1.409	90.71	0.191	12.30	13.56		34.43	
	Ration	27,667	0.798	220.83	0.129	35.72	16.17			

hay and 14.22 per cent of that of clover hay in non-protein form. Undoubtedly the stage of maturity is an important factor influencing the non-protein nitrogen content of hay. However, the small figure for clover hay in trial 1 seems unexplainable even on this basis.

The non-protein nitrogen content of corn silage, as shown in table 2, is exceedingly large. Annett and Russell (23) found 43.72 per cent of the total nitrogen of corn silage as non-protein nitrogen. They also showed that in the silo there was a loss of all nitrogenous constituents except non-protein, which gained 83 per cent. Shaw, Wright and Deysher (24)[4] found 61.99 and 61.41 per cent of the total nitrogen of corn silage as non-protein nitrogen. They also demonstrated strikingly that there was a great loss of albuminoid nitrogen in ensiling and a tremendous gain in non-protein nitrogen. That the stage of maturity greatly affected the non-protein nitrogen content of silage was clearly shown by Woodman and Amos (25).[5] Fifty per cent of the true protein was hydrolyzed in the case of very immature silage, while only 23 per cent was hydrolyzed in the case of fairly mature silage. These workers concluded that "the conditions of immaturity are favorable to the extensive splitting up of the protein of the crop into amino acids." The fairly large amount of non-protein nitrogen found in the silage used in the trials reported here is thus in accordance with previous experimental work.

While the grain mixtures used for the different cows in the same trial varied only slightly as to the per cent of total nitrogen, the per cent of total nitrogen as non-protein nitrogen varied considerably, from 6.41 per cent to 15.05 per cent in trial 1, from 9.10 to 18.01 per cent in trial 2, from 7.89 per cent to 11.44 per cent in trial 3, and from 7.30 to 19.08 per cent in trial 4. These variations could not be correlated with the make-up of the various grain mixtures, nor did any reasonable explanation for them present itself. Many of the analyses were repeated several times without changing the values first obtained.

The results given in table 2 and the data referred to in the discussion of these results must also raise questions as to the general usefulness of the published data on the non-protein nitrogen content of feeds. If the method itself is accurate and thus roughages may vary as greatly as the data show, tables giving the "true protein" content based on average analyses for non-protein must have a large probable error. That large variations may exist according to stage of maturity and other factors is reasonable to expect, as has been discussed. However, the data obtained for the grain mixtures raise a question as to the accuracy of the method itself—a question which limits the value of the following discussion and which should be borne in mind, although the extent of its effect cannot be evaluated.

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[4] Albuminoid method.

[5] Foreman alcohol titration method.

In terms of the rations fed, it was found that the timothy hay ration in every case, except for cow 2, trial 2, contained more non-protein nitrogen than did the clover hay ration (column 5, figures for "ration"). The per cent of total nitrogen as non-protein nitrogen was greater in every case on the timothy hay ration than on the clover hay ration (column 6, figures for "ration"). The per cent of total nitrogen of the ration as non-protein nitrogen contributed by the hay and silage was quite uniform in each trial (column 8), and was greater throughout on the timothy hay ration. While both types of rations furnished approximately the same amount of total protein, the timothy hay ration furnished the greater proportion of non-protein.

In column 9 data are given showing the non-protein nitrogen in the feces of each cow. Since these figures are in all cases very much smaller than those for non-protein nitrogen in the ration, as shown in column 5, it may be concluded that some at least of this non-protein nitrogen was digested. Digestion coefficients may be computed showing a digestibility of 60 to 80 per cent in most cases. Such figures cannot be considered accurate, however, both because there is no way of knowing to what extent this non-protein nitrogen in the feces originated from protein in the feed, and because some of the non-protein nitrogen in the feed may have been built into protein by bacteria yet not have been digested. It seems reasonable, nevertheless, to conclude that a large percentage of the non-protein nitrogen in the feed was digested, at least as well as the crude protein which was found to be from 55 to 65 per cent digestible by Maynard, Miller and Krauss (1).

Was this digested non-protein nitrogen utilized for the performance of any protein function? This cannot be proved, but there is presumptive evidence that it was:

The results reported by Maynard, Miller and Krauss (1) show a positive nitrogen balance in nine out of twelve cases where the digestible crude protein was generally insufficient to supply the maintenance requirement according to the Morrison standard plus the nitrogen in the milk. In view of this efficient utilization of digestible crude protein it seems reasonable to assume that the 15 to 20 per cent of it that was non-protein in nature was used to some extent at least.

Table 2 shows more non-protein nitrogen in the timothy ration than in the clover ration, yet according to Maynard, Miller and Krauss the nitrogen of the timothy ration was at least as well utilized as that of the clover ration.

Certain additional evidence is to be found in the discussion that follows of the partition of the nitrogen in the urine.

In table 3 data are presented showing the partition of nitrogen in the urine. Table 4 contains the same data expressed in per cent of the total nitrogen. The outstanding feature of these data is the large amount of undetermined nitrogen, showing that a relatively large amount of nitrogen is present in some form other than ammonia, urea, and hippuric acid. The same situation was found to exist in the work of Lindsay (26) in which, when only the urea and hippuric acid nitrogen are considered, 25.4 per cent of the total nitrogen remains as undertermined. In order to determine, if possible, the principal constituent contributing to the large amount of undetermined nitrogen, a sample of urine from a cow in another trial not reported in this paper, was analyzed for creatinine and creatine. Only a trace of creatinine was found, but 25 per cent of the total nitrogen was present as creatine nitrogen. Based upon Lindsay's work, in which 5.0 per cent of the total nitrogen in the urine was reported as creatinine, we would expect some creatinine to be present also. The reason

TABLE 3

Partition of nitrogen in the urine

TRIAL	COW	VOLUME EXCRETED	N PER 100 cc.	TOTAL N	AMMONIA N	UREA N	HIPPURIC ACID N	UNDETERMINED N
		cc.	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
1	1	7,518	0.8301	62.41	*	31.43	11.85	19.13
	2	10,460	0.6732	70.42	*	31.07	11.61	27.74
	3	7,523	1.1880	89.37	*	44.69	12.71	31.97
2	1	5,540	1.2524	69.38	*	27.87	14.39	27.12
	2	7,216	1.0406	75.09	*	38.10	12.15	24.84
	3	6,359	1.5226	96.82	*	48.46	14.37	33.99
3	4	5,508	1.0894	60.00	4.82	22.91	11.93	20.34
	5	5,079	1.0053	51.06	3.84	15.09	14.06	18.07
	6	5,860	0.9877	57.88	2.99	25.14	12.59	17.16
4	4	8,000	0.6575	52.60	9.20	8.08	11.18	24.14
	5	8,214	0.6138	50.42	2.83	20.62	10.90	16.07
	6	8,674	0.6474	56.16	2.43	21.95	10.45	21.33

* Not determined.

TABLE 4

Percentage partition of nitrogen in the urine

TRIAL	COW	TOTAL N	AMMONIA N	UREA N	HIPPURIC ACID N	UNDETERMINED N
		grams	*per cent*	*per cent*	*per cent*	*per cent*
1	1	62.41	*	50.35	18.99	30.66
	2	70.42	*	44.12	16.49	39.39
	3	89.37	*	50.00	14.23	35.77
2	1	69.38	*	40.16	20.74	39.09
	2	75.09	*	50.74	16.17	33.09
	3	96.82	*	50.05	14.84	35.11
3	4	60.00	8.03	38.19	19.88	33.90
	5	51.06	7.51	29.54	27.54	35.41
	6	57.88	5.16	43.43	21.76	29.65
4	4	52.60	17.49	15.36	21.26	45.89
	5	50.42	5.62	40.89	21.63	31.86
	6	56.16	4.33	39.08	18.61	37.98

* Not determined.

practically none was found may be that since creatinine is gradually changed to creatine when in contact with alkalies, and since cow urine is alkaline, the creatinine may have been hydrolyzed to creatine. This would also explain the large amount of creatine found. Presumably allantoin nitrogen was also present as a part of the undetermined nitrogen since Lindsay found 6.3 per cent of the total nitrogen in this form.

The tables indicate that while a greater volume of urine was excreted on the clover hay rations than on the timothy hay rations, there was no significant difference in the amount of total nitrogen and urea nitrogen excreted. Less total and urea nitrogen were excreted in trials 3 and 4 because the protein intake was less. The small amount of urea found in the urine of cow 4, trial 4, was probably due to the fact that this sample was improperly preserved, resulting in the breaking down of some of the urea into ammonia as indicated by the relatively large amount of ammonia found.

A small amount of ammonia was found. This is contrary to the results of Lindsay who found practically no ammonia. However, it is in accord with Salkowski (27) who found 0.38 gram of ammonia per liter in fresh cow urine. The differences in the amount of ammonia found in trials 3 and 4 are not great enough to be of significance.

It will be seen from table 3 that without exception slightly more hippuric acid nitrogen was excreted on the timothy rations than on the clover rations. While this suggests that a greater amount of benzoic acid and benzoic acid-like substances were present in the timothy rations, the difference is not great enough to be conclusive, as other factors, particularly bacterial decomposition of proteins, affect the amount of benzoic acid eliminated.

A study of the urine analyses indicates no difference in the protein metabolism on the two types of rations.

SUMMARY

Data are presented showing the distribution of non-protein nitrogen in dairy rations based upon clover and timothy hay, the digestibility of the non-protein nitrogen, and its utilization by cows in milk. The results of a study of the partition of the nitrogen in the urine collected while these rations were being fed are included. The following conclusions are based on these data:

1. The non-protein nitrogen in hay and silage varies greatly in different samples, presumably due to differences in stage of maturity, method of curing, and other factors. This large variation suggests that figures for true protein based on average analyses are of limited usefulness.
2. The albuminoid nitrogen method of the Association of Official Agricultural Chemists is of doubtful accuracy for distinguishing between crude and true protein in feedingstuffs.
3. Dairy rations made up of hay, silage, and grain, contain a relatively large amount of non-protein nitrogen. This nitrogen is apparently useful in meeting a part of the protein requirement of a cow in milk.
4. There is no difference in the protein metabolism when timothy hay or clover hay is fed, as indicated by urine analysis.

The writer desires to express his appreciation for the generous assistance and valuable suggestions given by Dr. L. A. Maynard, both in conducting the experimental work and in preparing this paper. He also wishes to express his appreciation to Dr. J. B. Sumner for his valuable suggestions as to methods for urine analyses and for the use of his laboratory, and to Dr. R. C. Miller for his generous assistance in conducting the experiment.

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THE CREAM PLUG*

ITS CAUSES AND PREVENTION

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One of the problems met in the selling of bottled cream is the formation of the cream plug. This cap or plug which frequently gathers on the cream varies in tenacity from the consistency of heavy cream to a leathery condition of such firmness that it prevents the spilling of the cream when the bottle is inverted. The undesirability of such cream plugs is self-evident. A waste of butter fat occurs when they are discarded as they usually are; but from the milk dealers' standpoint the chief objection is the unfavorable impression such bottled cream makes on the consuming public.

Two attempts to gather information on this problem have been made in the form of questionnaires sent to milk dealers. These surveys of opinions served to emphasize the importance of the problem, but contributed little to its solution, for they failed to reveal the fundamental factors involved in it. In the experimental work here reported, the main aim was to determine these fundamental factors.

THE COMPOSITION AND MICROSCOPIC APPEARANCE OF CREAM PLUGS

In order to determine the exact nature of the cream plug, analyses and microscopic examinations were made. The plugs from two sources were analyzed; some were taken from returned University cream and others from the cream of a local company. These plugs were analyzed for fat by ether extraction and tested for total solids, by drying to constant weight at 100°C.

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The analyses of the cream plugs show that they consist principally of fat. From our knowledge of the normal rising of fat in milk, we would hardly expect such a rich accumulation of fat if the fat were still in normal emulsion. Microscopic examination of the cream plugs readily revealed that the fat was no longer in normal emulsion but had coalesced into irregular masses of fat. (Compare figures 1 and 2.)

TABLE 1

The composition of cream plugs

SAMPLE NUMBER	PER CENT FAT	PER CENT SOLIDS-NOT-FAT	PER CENT WATER
1	77 60		
2	79 80		
3	62 05	4 49	33 46
4	65 07	2 10	32 83
5	55 40	5 30	39 30
6	58 10	4 10	37 80
7	61 40	4 00	34 60
Average . . .	65 57		
Average excluding 1 and 2	60 40	4 00	35 60

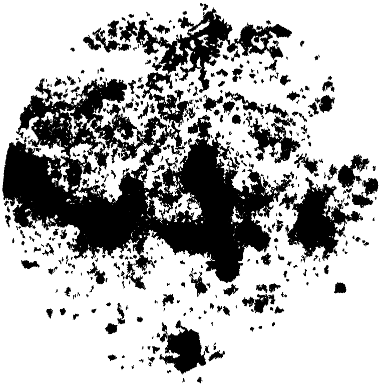

FIG. 1. LOW POWER PHOTOMICROGRAPH OF CREAM PLUG, 70 PER CENT FAT

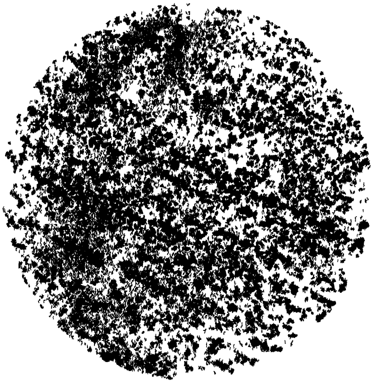

FIG. 2. LOW POWER PHOTOMICROGRAPH OF CREAM, 70 PER CENT FAT

THE FUNDAMENTAL CAUSE OF CREAM PLUG FORMATION

From the general knowledge that emulsions are less stable as the size of the globules increases and that the large globules rise more rapidly than small globules, the logical deduction is that the formation of cream plugs is dependent upon the size of the fat globules. The large fat globules rise more rapidly and are more likely to coalesce than the small fat globules. A more exact understanding of the relative velocities with which globules of different sizes rise, can be obtained by an application of Stokes equation. Ruling out those factors in this equation which are constant or over which we have no control, we find that the velocity with which the globules rise is directly proportional to the square of their radius, and inversely proportional to the viscosity of the liquid through which they are rising. Thus the size of the fat globules and the viscosity of the serum of the cream may be expected to be factors in cream plug formation.

Observations made in the course of the experimental work are in harmony with these deductions.

FACTORS THAT AFFECT THE SIZE OF FAT GLOBULES

Since the size of the fat globules determines whether the cream will develop cream plugs, it becomes of importance to consider the factors that affect the size of the fat globules. The effect of breed and stage of lactation on the size of the fat globules in the milk are well known. Similarly, the possibility of partial churning of milk while being transported especially in partly filled cans and the tendency of freezing to collect the fat, need only be mentioned here. No further experimental proof is required on these factors.

The experimental work conducted concerned itself mainly with demonstrating the effect of various types of agitation to which the milk and cream may be subjected in the milk plant.

METHOD OF MEASURING THE SIZE OF CREAM PLUGS

To measure the tendency of the various experimental cream samples to form plugs, the weight of the plug was obtained.

This was considered satisfactory since the toughness of the plug was in general proportional to the weight.

A number of special cups were made of perforated sheet metal as shown in figure 3. One of these cups was placed into each bottle of cream immediately after bottling. After the bottled cream had been stored as desired the plug was then removed by slowly raising this cup out of the bottle by means of the handle which projected slightly above the surface of the cream. The cup was allowed to drain a standard length of time, and the weight of the material retained in it was then determined.

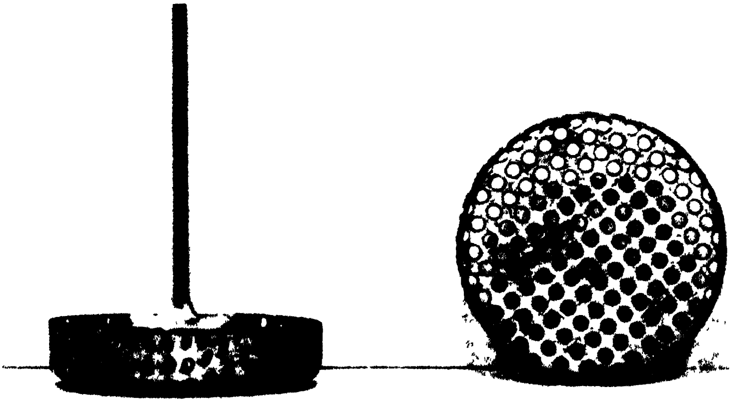

FIG. 3. CUP USED IN REMOVING CREAM PLUGS

THE EFFECT OF AGITATION

In order to demonstrate the effect of agitation on cream plug formation, 25 per cent cream was agitated at 45°F. by pouring from one pail into another 25 times. Samples of this agitated cream and from another portion of the same cream that had not been so agitated were set aside as described above, and after forty-eight hours the weights of the cream plugs were determined (table 2). Samples of the fresh agitated and unagitated cream were also taken for microscopic examination. Photomicrographs were taken of these samples in a 1:50 dilution in neutral 3 per cent gelatin (fig. 4).

The results obtained demonstrate that the agitation caused the fat to collect into larger globules and increased cream plug formation.

TABLE 2

The effect of agitation on cream plug formation

WEIGHTS OF CREAM PLUGS FROM:	
Agitated cream	Unagitated cream
grams	*grams*
5.5	5.5
7.1	3.9
6.9	4.3
6.0	4.8
6.6	3.2
Average......6.42	3.94

TABLE 3

The effect of bottling foamy cream on cream plug formation

Cream agitated, poured 25 times

WEIGHTS OF CREAM PLUGS FROM:	
Foamy cream	Air removed by vacuum
grams	*grams*
14.3	6.6
13.8	6.5
13.4	7.2
13.0	7.1
15.7	4.9
11.8	5.5
11.6	7.6
14.5	8.1
12.5	7.5
11.7	7.4
Average......13.2	6.8

In another similar experiment the agitated samples (37 samples) averaged 2.26 grams heavier cream plugs than the unagitated samples (39 samples).

In these two experiments where cream was agitated cold by pouring it was necessary to eliminate any air that was incorporated, by subjecting the cream to a vacuum for five minutes, as preliminary trials had shown that if any foam was not eliminated a foamy accumulation would interfere with the measurement of the true cream plug.

Since under commercial conditions a similar condition might at some time be encountered, the results obtained when the foam was not eliminated, are given in table 3. The cream used was unpasteurized 25 per cent cream, agitated by pouring from one pail to another 25 times. The foamy plugs were not so close in texture and not so firm as the usual plugs, but nevertheless they were objectionable.

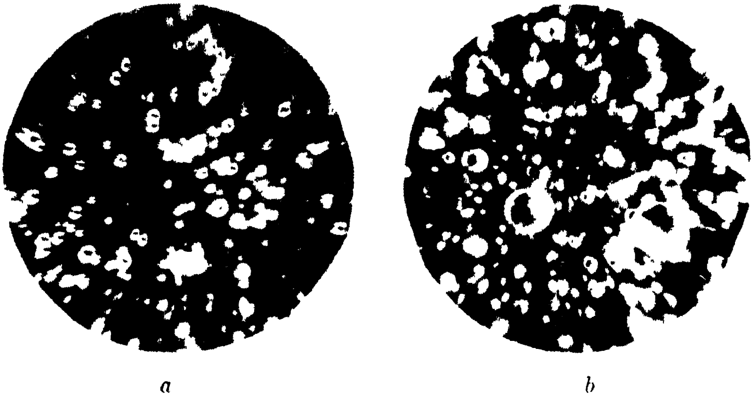

FIG. 4. *a*, PHOTOMICROGRAPH OF NORMAL CREAM, *b*, PHOTOMICROGRAPH OF AGITATED CREAM

THE EFFECT OF AGITATION AT DIFFERENT TEMPERATURES

In order to determine at what temperatures the effect of agitation is most pronounced, a series of experiments was conducted similar to the above, using cream heated to temperatures of 50°, 100°, 120° and 140°F. Air was eliminated as in the other experiments. The results given in table 4 show that agitation at the lower temperatures is most harmful.

That agitation at pasteurizing temperature has little harmful effect is further demonstrated by the results given in table 5 where the weight of the plugs is given for samples of cream taken from a coil vat pasteurizer at three different intervals,—immediately after the cream had been heated to 145°F., after having been held at 145°F. for twenty minutes, and after thirty minutes.

TABLE 4

The effect of agitation at different temperatures on cream plug formation

EXPERIMENT NUMBER	WEIGHTS OF CREAM PLUGS FROM CREAM				
	Agitated at 140°F.	Agitated at 120°F.	Agitated at 100°F.	Agitated at 50°F	Not agitated
I	3.8	3.1	4.1	5.6	3.0
	3.1	3.4	4.7	5.0	3.2
	2.6	2.9	3.4	6.5	2.4
	2.8	4.5	3.4	4.6	2.9
II	3.9	3.1	4.6	5.9	3.3
	3.3	4.5	3.4	5.2	3.5
	3.9	4.0	4.0	5.2	1.9
	3.6	3.6	4.3		
Average	3.4	3.7	4.0	5.5	2.9

TABLE 5

The effect of agitation at pasteurizing temperature on plug formation

Stored three days at cooler temperature

WEIGHTS OF CREAM PLUGS.		
Cream heated to 145°F.	After 20 minutes agitation at 145°F.	After 30 minutes agitation at 145°F.
2.6	3.3	3.1
2.9	5.0	4.0
3.0	5.0	4.9
3.5	4.6	4.3
3.0	4.0	3.8
Average.....3.0	4.4	4.0

The effect of agitation at lower temperatures is further demonstrated in an experiment in which a lot of cream was divided into two lots; one lot was pasteurized and cooled in a coil vat pasteurizer; the other lot was heated to 145°F. by means of a flash pasteurizer, held at 145°F. for thirty minutes and then cooled over an aerator. The former of these two lots of cream was thus subjected to considerable agitation in the heating and cooling, while in the latter lot this agitation was reduced to a minimum. The results given in table 6 show that reducing the agitation to a minimum while heating and cooling the cream materially reduces cream plug formation as compared with heating and cooling as in a coil vat pasteurizer.

TABLE 6

The effect of reducing agitation to a minimum while heating and cooling

WEIGHTS OF CREAM PLUGS FROM CREAM	
Pasteurized and cooled in coil vat	Flashed to 145°F., held 30 minutes, cooled over aerator
9 0	3 0
9 1	2 8
9 6	4 5
6 7	3 5
7 0	4 1
9 3	4 2
8 6	3 8
9 3	4 5
8 6	4 3
8 3	4 1
8 5	4 0
9 7	5 1
9 8	3 6
8 7	5 0
9 5	4 4
Average..... 8 78	4 06

A consideration of the results given in tables 4, 5, and 6 leads to the conclusion that agitation of cream at any temperature tends to increase cream plug formation, and that agitation of cream at lower temperatures favorable to churning is decidedly more harmful than agitation at pasteurizing temperatures.

THE EFFECT OF AN UNBALANCED SEPARATOR BOWL IN SEPARATING THE CREAM

The separator bowl being out of balance is frequently mentioned as a cause of cream plug formation. In order to determine whether the vibration and possible agitation to which cream would be subjected in an unbalanced separator bowl would have any effect on cream plug formation, an experiment was conducted in which a lot of milk was divided into two lots,—one lot was separated by means of a cream separator with the bowl running true, and the other lot was separated by the same separator after the bowl had been unbalanced by placing a small sheet of lead between two of the plates in the separator bowl. The two lots of cream were separately standardized, pasteurized and bottled. The results of two such experiments are given in table 7. The fat test of the skim milk in each case is given as an indication of the fact that the bowl was unbalanced. The results demon-

TABLE 7

The effect of an unbalanced separator bowl on cream plug formation

EXPERIMENT NUMBER	WEIGHT OF PLUG	
	Unbalanced bowl	Balanced bowl
	Skim milk test 0.13 per cent	Skim milk test 0.075 per cent
I	3.0	2.6
	2.0	2.1
	2.4	1.2
	2.0	2.0
	1.7	1.7
	1.7	2.2
	Skim milk test 0.118 per cent	Skim milk test 0.042 per cent
II	4.0	3.0
	2.6	3.4
	3.5	4.0
	3.5	3.2
	3.7	3.7
	4.6	4.6
Average	2.9	2.8

strate that the presence of a cream plug on bottled cream cannot be attributed to an unbalanced separator bowl.

THE EFFECT OF STORAGE TEMPERATURE ON CREAM PLUG FORMATION

In considering the fundamental causes of cream plug formation it was pointed out that two important factors that determine the rise of fat globules are the size of the globules and the viscosity of the serum. In the foregoing the effect of the size of the fat globules has indirectly been considered as affected by agitation of the cream.

TABLE 8

Temperature of storage and cream plug formation

Held four days, 20 per cent cream

	WEIGHT OF PLUG	
	Room temperature 70°F	Cooler temperature 42°F.
	grams	
	15.10	6.10
	14.00	7.37
	17.95	5.37
	17.69	6.17
	15.85	4.62
	11.17	7.53
	18.07	6.36
	8.75	5.30
	18.67	5.20
	17.10	4.68
	15.40	5.43
	17.92	3.35
	17.87	5.75
	19.57	5.27
	15.75	6.81
	20.94	9.48
Average......	15.94	5.92

The viscosity of the serum as a factor in this problem is controllable only in so far as temperature affects the viscosity. It is commonly known that the viscosity of fluids increases as the temperature is lowered. It is to be expected therefore that storing the cream at low temperatures would reduce the rise of the globules and reduce cream plug formation.

In an experiment to demonstrate the effect of storage temperatures, pasteurized 20 per cent cream was bottled and bottles stored at 42° and 70°F. for four days. The weights of the plugs formed were then determined. While there had been a noticeable increase in the acidity of the cream at 70°F. the cream had not thickened, so that the results given in table 8 represent the weight of the actual plugs (see fig. 5). At 70°F. plugs were formed that were very tough and leathery and were approximately three times as heavy as those formed at 42°F. That this difference in the weight of plugs at the two temperatures is to be attributed to the physical effect of the temperature difference and not to the bacterial growth and acidity differences was demonstrated in experiments in which it was found that there was no difference in cream plug formation at room temperature between samples of different acidities and between preserved and unpreserved samples.

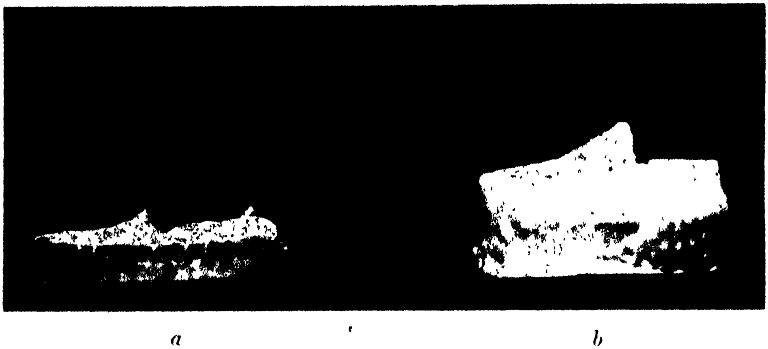

FIG. 5. *a*, SHOWS AVERAGE CREAM PLUG FORMED AT COOLER TEMPERATURE; *b*, SHOWS AVERAGE CREAM PLUG FORMED AT ROOM TEMPERATURE

EVAPORATION AND CREAM PLUG FORMATION

It has been suggested that cream plug formation may be due to evaporation from the surface of the cream. The analyses of the cream plugs lend some strength to this suggestion, although the results given in table 1 are not extensive enough to warrant drawing any positive conclusion on this point. Furthermore, even if these results were accepted as conclusive, the high solids-not-fat content might be due largely to adsorption.

In order to determine to what extent evaporation might be a factor in cream plug formation a comparison was made in which thirty bottles of cream from the same lot were divided into three lots and capped as follows: Lot 1 was capped with the usual cap and further sealed by carefully paraffining over the cap; lot 2 was capped with the usual cap only; and lot 3 was covered with two thicknesses of porous paper toweling held in place with rubber bands. Each of these three lots was subdivided into two lots of five bottles each and stored at room temperature and at the cooler temperature (40° to 45°F.). After two days the cream plugs were determined in the usual manner. The results given in table 9 show that evaporation was only a slight factor. In-

TABLE 9

The effect of evaporation from capped bottles on cream plug formation

	WEIGHT OF PLUGS		
	Paraffined cap	Usual cap	Porous paper cap
	grams	*grams*	*grams*
Room temperature storage; time, two days	15.5	14.5	16.2
	13.1	14.8	15.8
	15.4	14.0	15.7
	13.0	15.6	15.5
	12.8	13.3	16.2
Average	13.96	14.44	15.88
Cooler temperature storage; time, two days	6.6	6.4	5.6
	6.6	5.7	7.0
	5.5	6.7	6.8
	6.1	4.4	6.5
	5.4	3.9	7.0
Average	6.04	5.42	6.58
Grand average	10 00	9.93	11.23

cidentally these results again emphasize the importance of storage temperature in preventing cream plug formation.

REMEDIES FOR CREAM PLUG FORMATION

The above results and discussion indicate that the prevention of cream plug formation resolves itself into handling the milk and cream in such a manner as to prevent the fat from coalescing into large globules and clusters of fat. In practical operations this means reducing agitation of the milk and cream to a minimum, especially at temperatures that are favorable to churning. While it is impossible to enumerate all objectionable practices a few of the more common ones are as follows:

1. Transporting milk in partly filled cans. The extent of churning taking place will depend upon the amount of shaking to which the cans are subjected in transit and the temperature of the milk. Milk improperly cooled, at temperatures of 60°, 70° and even 80°F. will churn more than milk properly cooled at 50°F., or lower.
2. Freezing of the milk. This is known to cause the partial coalescence of the fat.
3. Delayed heating of the cream in a coil vat pasteurizer. In some instances the cream may be run into a coil vat, agitated with the coil and not promptly heated to pasteurizing temperature. This is injurious since any delay means that the cream will be subjected to more agitation than necessary and this is at a low temperature, which is most harmful.
4. Running the coil too rapidly.
5. Having the coil vat pasteurizer only partly full. The agitation in such cases is more severe than in a full pasteurizer.
6. Cooling the cream in the vat pasteurizer with the coil. In all cases cooling by running the cream over an aerator or through an internal tubular cooler is preferable because of the decidedly smaller amount of agitation involved.
7. Repasteurizing of the cream.

In addition to the practices mentioned above and which concern themselves with the coalescing of the fat into larger masses, the storage temperature of the bottled cream must be mentioned as important. The higher the storage temperature, the less viscous the serum of the cream will be, and the more rapid and the more pronounced the cream plug will be.

If the milk and cream is obtained from such sources that the milk dealer has little or no control over the past history of these products, or where the exercising of the proper control from the standpoint of cream plug formation is difficult or impossible, remedies other than preventative must be employed. In such cases cream plugs can still be eliminated by applying the knowledge that the fundamental factor is the presence of large fat globules, by reducing the size of the fat globules by means of the centrifugal emulsifier or by means of the homogenizer.

By experiment it was found that the emulsifier and the homogenizer would eliminate the undesirable cream plugs. In using the homogenizer it is to be remembered that the higher pressures will reduce the whipping quality of the cream and increase the tendency of the cream to "feather" when added to coffee or other hot liquid. Pressures should be used that are just sufficient to accomplish the desired result.

The exact pressure necessary depends upon the condition of the cream, the make and mechanical efficiency of the homogenizer. Pressures ranging from 200 to 1000 pounds per square inch are being used for this purpose.

SUMMARY

The cream plugs contain a high percentage of fat and microscopic examination shows that the fat has coalesced into irregular masses. The fundamental cause of cream plug formation is the presence in the cream of large fat globules which will rise rapidly into a dense layer and coalesce. This rising and coalescing of these fat globules is most rapid at higher storage temperatures because of the fact that the serum through which the globules must rise is less viscous.

The prevention of cream plug formation, therefore, resolves itself into handling the milk and cream in such a manner that partial churning is avoided, or if this is impossible, reducing the size of the fat globules by either emulsifying or homogenizing at low pressures. Storing the cream at proper storage temperatures is helpful in preventing the formation of the cream plugs, but this is beyond the control of the milk dealer after it leaves his plant and, therefore, should not be relied upon to any great extent.

MAINTENANCE REQUIREMENTS FOR CALVES TESTED BY LIVE WEIGHT METHODS*

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In connection with our studies of growing cattle the question of the requirements of the animal for maintenance has often arisen. Armsby (1) is the only authority who has published tables of estimated maintenance requirements including ranges of weights normal to calves under one year of age. The figures for the smaller weights were calculated by using his extensive results from mature animals. The question of the accuracy of these figures first arose as the result of an experiment in which a heifer made a gain of approximately a pound per day for six months while receiving a ration, the net energy value of which, when calculated by the Armsby method, was approximately the amount set forth as the maintenance requirement in his tables.

The limited value of data professing to deal with maintenance requirements taken by the live weight method is fully recognized especially when the subjects are immature animals. However, it is believed the data to be presented have some value as an indication of the extent to which maintenance figures calculated from mature animals are applicable to young animals.

PROCEDURE

Three procedures might be used in a study of this problem. The first is to feed an animal a given percentage of the energy estimated as the maintenance requirement, using Armsby's maintenance figures and method of calculating energy values.

The second is to vary the feed from day to day as appears necessary to maintain a uniform weight for a period of time with a determination later of the energy used during the trial.

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The third is to feed a constant ration which has been computed from a preliminary period to supply sufficient energy for maintenance. If the estimate is too low the animal will lose weight until the ration is sufficient for maintenance. If too high, the animal will gain in weight until an equilibrium is reached. Experience indicated the third plan to be most satisfactory and it was used exclusively during the latter part of the study.

The first plan required that the ration be adjusted at frequent intervals to allow for any change in the weight of the animal. Variations from day to day in weight due presumably to fill often result when this plan is followed in changes being made in the ration that later are found to have been a mistake. The same difficulty of knowing the true weight of the animal interferes with the details of operating the second plan. The third plan of feeding a constant ration throughout the maintenance period eliminates some of these difficulties and if the ration chosen is close to the true maintenance requirement the animal will soon adjust itself to it by a gain or loss in weight and thereafter remain quite constant.

It is recognized that with mature animals the length of the trial is an important factor regarding the dependability of the results. With growing animals it is not advisable to undertake to keep the animal at a constant weight for any length of time. Waters (2) found that profound physiological changes occur in young animals that are fed a limited ration. With animals of this age the live weight may readily remain the same while the animal is not in energy equilibrium. Experience indicated that about forty days was the most satisfactory period for use with the young calf while for older animals longer periods are feasible.

ANIMALS AND RATIONS USED

Fifteen animals were used, all of the Holstein breed, of which five were males and ten females. The males were used in the lighter weights only and on the plan where a constant ration was used. Alfalfa hay and wheat straw were used for roughage. In order to keep a growing animal quiet on a limited ration, experience indicated it was necessary to feed a bulky ration which will insure a normal amount of material in the alimentary tract. To secure the desired bulk with alfalfa alone would in some cases provide more energy than was desired. By using wheat straw as a low net energy filler it was possible to make such adjustments as necessary in energy intake and still keep the animals reasonably contented. The grain ration used was uniformly a mixture of ground corn 4 parts, wheat bran 1 part, linseed oilmeal 1 part.

In making the advance calculations of the ration to be fed average values were used in most cases as the chemical analyses were generally not available until later. All feeds used except the skimmilk were analyzed by the Division of Agricultural Biochemistry. From these data the net energy values were calculated according to Armsby's method. The digestion coefficients as given by Morrison (3) were applied to the chemical analyses. Factors given by Armsby (1, p. 650) were used for the calculation of the therms metabolizable energy. The factors for the increment of heat production were those recommended by the same author (1, p. 652). A protein supply was provided at all times considerably above the amount usually specified as the minimum required for maintenance.

The animals were kept in individual stalls bedded with shavings and allowed the freedom of a dry lot a part of the day, when the weather was not too severe. Water was offered once daily in the barn and was available in the exercise lot. Salt and bone meal were available in each stall. Each animal was weighed daily before the morning feed.

EXPERIMENTAL

Experiment 1. Three animals approximately six months old at the beginning were used in this trial. An attempt was made by frequent adjustments to provide 100 per cent of the Armsby maintenance requirement. However, as the calculations for adjusting the ration were made by using average analyses of the feeds included and the analyses later available showed somewhat more than average value the ration actually received was a trifle higher than intended. The results are shown in figure 1. The animal E-12 received 101.6 per cent for the 130 days, E-14, 101.4 per cent and E-17, 101.9 per cent of Armsby's standard for the 115 days on experiment. Two of the animals gained about half a pound daily and the third somewhat more.

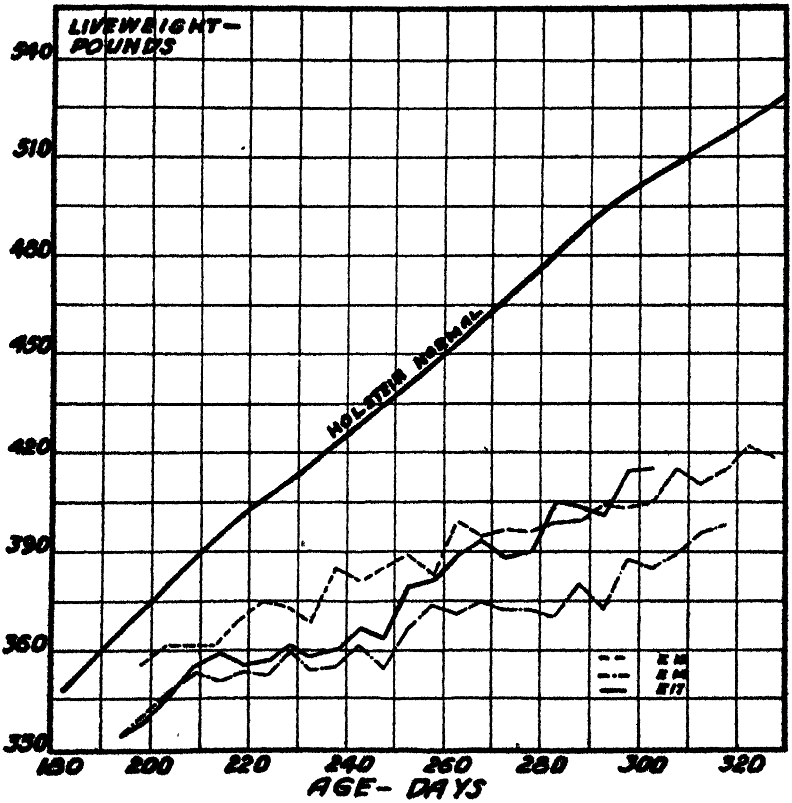

Fig. 1. Results from Supplying Calves with 100 Per Cent Net Energy Needed for Maintenance According to Armsby's Tables

The three heifers receiving a net energy intake, as prescribed by Armsby, gained over half a pound daily for the period of the trial, 110 to 120 days.

These results confirmed our previous experience and indicated the energy for maintenance of small animals as specified by Armsby's tables is unnecessarily high when calculated by the present net energy values.

Experiment 2. Four animals were used ranging in age from 100 to 271 days at the beginning of the experiment. An attempt was made to keep the weights of the animals constant by adjusting the rations at frequent intervals although the results were only partially successful. The fact that in every case, although the ration given was below 100 per cent of Armsby's standard, all the animals maintained their weight or made gains indicated the Armsby standard calls for more energy than these animals required.

Experiment 3. In this experiment the third plan of procedure already described was followed—that of estimating on the basis of a preliminary period what feed intake would be necessary for maintenance then continuing to feed this ration without change throughout the period. If the ration as estimated proved to be more liberal than maintenance a gain would presumably be made until equilibrium was established. If too small the same adjustment would occur at a point somewhat below the original weight.

Data were taken from 21 periods using 11 different animals. In those cases where the animal was used twice the first period was followed by one of liberal feeding and the second trial was not undertaken until the animal was again approximately normal in weight for its age. In all cases a preliminary period was used for adjusting the ration to a point which appeared to result in a stabilization of weight. However in some cases the amount of feed necessary to maintain the animal was misjudged during the preliminary period and as the plan followed was to make no changes in ration during the trial period gains or losses in certain cases were too much to justify the use of the data obtained. In table 1 is presented a summary of the results from 18 periods taken with 11 individuals in trials where the weight remained essentially the same. The first 16 represent animals in normal condition of flesh and the last two animals in very high condition. These data show that all calves in condition described as normal were maintained on a ration averaging 90 per cent of the Armsby specification. In only one of the 16 trials was 100 per cent of Armsby's maintenance figures reached and this is apparently accounted for by a condition of the animal above normal.

Condition as a factor. The last two figures in the table give the results of a maintenance period of two calves in high flesh at the time. It will be noted in both cases the requirements were decidedly above Armsby's figures.

TABLE 1

Summary of all trials resulting in weight maintenance

NUMBER OF ANIMAL	LIVE WEIGHT		LENGTH OF TRIAL	NET ENERGY RECEIVED PER DAY	NET ENERGY PER 1000 POUNDS	PLANE OF NUTRITION	CONDITION OF ANIMAL
	Initial	Gain for period					
	pounds	*pounds*	*days*	*therms*	*therms*	*per cent*	
E-79	87	0	20	1.16	5.91	98 5	Normal
E-87	117	−3	25	1 33	5 56	92.7	Normal
E-79	135	0	25	1 41	5 36	89 3	Normal
391	233	−1	40	1 75	4 62	77 0	Normal
390	249	−2	45	1 94	4 90	81 7	Normal
391	275	4	35	2 29	5 41	90 2	Normal
390	308	1	15	2 48	5 44	90 7	Normal
E-15	323	2	35	2.55	5 42	90 3	Normal
E-16	394	0	40	2 94	5 47	91 2	Normal
E-65	416	−1	35	3 11	5 58	93 0	Slightly high
E-55	455	0	40	3 20	5 41	90 2	Normal
E-54	468	2	40	3 21	5 32	88 7	Normal
E-65	537	0	25	3.15	4.77	79 5	Thin
E-53	663	1	25	4.26	5 60	93 3	Slightly high
E-46	798	1	40	5 18	6 02	100 3	Above normal
E-53	861	2	35	5 48	6 06	101 0	Above normal
Average........		375	32 5		5 43	90.5	
E-55	257	1	25	2 84	7 03	117 1	Very fat
E-54	284	−2	40	2 83	6 55	109 1	Very fat
Average.........			32.5		6 79	113.1	

First group of 16: mean average therms net energy required per 1000 pounds, 5.43 ± 0 066; standard deviation 0.390 ± 0 046.

Including all, mean average therms net energy required per 1000 pounds. 5.58 ± 0.090; standard deviation 0.569 ± 0 .064.

These two animals had been on a high plane of feeding, including whole milk, previous to the experimental period and were as a result in a high condition of flesh. This condition is further shown by the fact that E-55 was 22.4 per cent and E-54, 33.6 per cent above normal weight at the beginning of the trial.

One of these heifers as shown by the figures in table 1 required 109 and the other 117 per cent of Armsby's estimated energy requirement to maintain weight. The evidence that condition is the factor responsible is further strengthened by the results of using the same animals later. The figures in the same table show that at an age of about nine months the same animals were maintained with a ration supplying 89 per cent of Armsby's requirement which is in line with the results from the others.

GENERAL DISCUSSION

It is possible the ability of the dairy calf to maintain itself on a ration somewhat below Armsby's calculated amounts is to be attributed to the use in his experiments of animals of the beef type, normally in higher condition than dairy type animals. The evidence presented in this report indicating the importance of condition corroborates much of the evidence on the subject as summarized by Armsby and Moulton (4). Furthermore the results reported in the present paper are in line with the recent results of Cochrane, Fries and Braman (5) regarding the energy requirement for maintaining three Jersey cows. Two mature cows were maintained on approximately 90 per cent of the Armsby figure while one especially quiet animal used only about 70 per cent.

The revision of the net energy value now under way as announced by Forbes will affect the calculations of these trials. Alfalfa furnished a portion of the nutrients in nearly all the rations and assigning a higher value to alfalfa will raise the plane of nutrition beyond that calculated in this study. A recalculation will be desirable after the revision is completed.

One factor of economic importance indicated by this study is the increased energy required for maintaining growing heifers in high condition. On the other hand care should be taken not to go to the other extreme lest the animals be underfed to the extent of affecting the size adversely.

CONCLUSIONS

The net energy (calculated by the Armsby method from the chemical analyses of feed used) necessary to maintain uniform weight in dairy bred calves in normal flesh is about 90 per cent of that set forth in Armsby's Tables.

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A STUDY OF "FLAKY" MILK*

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It has become the practice in certified dairies to examine the milk from each cow before each milking. The usual procedure consists in drawing three streams from each teat upon a brass screen containing 100 meshes to the inch. If the milk passes readily through the screen and no residue remains, the secretion is regarded as normal; if however particles or floccules remain on the screen, the milk is rejected. Thus gross abnormalities in the secretion are readily detected, since the presence of flocculi is regarded as one of the principal signs of udder derangement. In a considerable number of instances floccules in small numbers are observed in the milk but no palpable abnormalities are found in the mammary gland. The general appearance of the secretion from such cases is not greatly altered except for the presence of relatively few, irregular, elongated, tiny, white particles. The floccules may be present for a single milking only or they may occur during a few days, disappear, and recur after varying intervals. That the condition is of considerable economic importance is borne out by the fact that a large number of cases occurred in a large herd during 1926, necessitating a considerable loss of milk to the producer. The condition is of considerable interest from the standpoint of the public health, since slight abnormalities in the character of the milk afford the most practicable and most rapid method for the detection of udder disease. Hence a knowledge of the true nature of the particles and their significance is essential. With these points in view the milk and the udders of a number of cases were examined.

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METHODS

At the start we examined the milk from the involved quarters of 20 consecutive cases as they occurred in a large herd. They were those reported to the herdsman by the foremilker. The samples for examination were obtained by one of us before the next regular milking. The udder was cleansed and the milk was drawn directly from the udder and immediately brought to the laboratory for examination. A mixed sample from the uninvolved quarters was usually examined. The procedure employed was to dilute 1 cc. of the milk with 9 cc. sterile NaCl solution. 0.5 cc. of the mixture was then plated with 0.5 cc. defibrinated horse blood and 10 cc. agar. Ten cubic centimeters of the milk was centrifuged rapidly for 10 minutes and blood agar slants in series inoculated wih a large loop of the sediment. Films were also prepared from the sediment. The plates were examined after twenty-four and forty-eight hours incubation. The fresh milk was always poured through a soil sieve of 100 meshes to the inch and any residue washed gently with NaCl solution. Particles were thus rendered easily visible and could be picked from the screen and examined microscopically. In addition quantities of milk varying from 25 to 50 cc. from 13 cases were centrifuged at high speed and the sediment, after suspending in sterile broth, injected into the peritoneal cavities of young guinea pigs. A sample was saved and when possible coagulated with rennet and the resulting whey, or the whole milk, tested for B. abortus agglutinin. The brief history given with the protocols of the cases was obtained from the herd records after the case had been studied. Inasmuch as the general features of the condition are similar only a few cases will be mentioned in detail.

STUDY OF CASES

Case 1425. The milk from the left hind quarter was reported to contain flakes on January 5, 1927. Abnormalities of the quarter were not noted. The milk contained large numbers of tiny, elongated, white particles composed of leucocytes and mucus, and others made up of epithelial cells. The centrifuged sample revealed considerable yellow, tenacious sediment composed largely of leucocytes in masses. The milk contained 2200 bacteria per cubic centimeter; 95 per cent of them were hemolytic staphylococci. The agar slant cultures from the sediment developed hemolytic staphylococci. The milk from the other three quarters appeared normal and failed to contain the staphylococci.

Case 1426. On July 13, 1926, mastitis developed in the right hind quarter. On August 1 and 2 and December 21, 26, and 27, the milk from the left front quarter was flocculent. Mastitis developed in this quarter on February 16, 1927. On December 22, 1926, the quarter appeared slightly firmer along the ventral aspect. The milk contained small floccules composed of sheets of epithelial cells. On December 27, there were a considerable number of elongated flocculi composed of leucocytes and mucus. There was considerable sediment made up of leucocytes. Micrococci were found in the films both free and within phagocytes. The plate cultures revealed 9540 hemolytic staphylococci per cubic centimeter. The same organism had been found on December 22. It was also present in the attack of mastitis on February 16, 1927.

Case 1427. Abnormalities in the milk from the left fore quarter were noted March 9, November 30, and December 11, 1926. On February 4, 1927, clinical mastitis developed in this quarter. Examination of the quarter on December 12, 1926, failed to reveal abnormalities. The milk on that day contained small flakes of epithelium. It was more watery in appearance than that from the other quarters. There was considerable yellow sediment composed of large numbers of leucocytes and cells of the endothelial type. The plate revealed 1000 non-hemolytic streptococci per cubic centimeter in the quarter. The milk from the other three quarters did not contain the streptococcus nor was an abnormal amount of sediment noted in the centrifuged sample.

Case 1428. On January 9, 1927, the milker reported flocculi in the milk from the left fore quarter. The sample on January 10 failed to show flakes. Plate cultures revealed 3840 B. lactis aerogenes per cubic centimeter. The centrifuged sample revealed an excessive amount of sediment composed of masses of leucocytes. On January 11, clinical mastitis developed, the milk was purulent, and the bacilli were isolated from the plates.

ANALYSIS OF THE FINDINGS IN 20 CASES

In our series of 20 cases the bacteriological findings were as follows:

From 9, non-hemolytic streptococci
From 5, hemolytic streptococci
From 4, hemolytic staphylococci
From 1, B. lactis aerogenes
From 1, nothing significant

As a rule the organisms were in moderate numbers, rarely above 9000 per cubic centimeter. In most instances udder pathogens appeared in blood agar plate cultures containing 0.05 cc. of milk. However, in some cases relatively few organisms grew in the plates, but the blood agar slant cultures prepared from the sediment revealed a type capable of giving rise to udder disturbance. In one instance neither the plate cultures nor the horse blood agar slants revealed a suggestive organism. However, sufficient time had elapsed between the attack and our examination to warrant the presumption that any pathogen may have been completely eliminated from the udder.

In 7 cases it was possible by palpation to detect abnormalities in the quarter. They consisted of irregular, firm areas, either diffuse or sharply circumscribed, in the region of the cistern or about the larger ducts. In 13 we failed to find such abnormalities. Five cows of the series developed mastitis subsequent to our examination.

It is of further interest to note that 7 cows had no previous history of disturbance in the quarter involved prior to our examination; 4 had two attacks, 1 three, 3 four attacks, 3 others had 5 attacks, 1 had been reported six times, and another on ten occasions.

The floccules observed on the sieve vary considerably in appearance and number. Those apt to arouse the attention of the fore milker are usually elongated, verminous-like particles, tenacious in character. In general they are 1 or 2 mm. in length, although at times some may reach a length of 5 mm. When these are washed free from casein, they are found to be composed of densely packed masses of leucocytes, apparently held together by mucus. As a rule they are covered with fat globules, but when the particle is crushed between coverslip and slide and examined microscopically its true nature is apparent. Their tenacious character and castlike appearance are characteristic. Particles of this nature were readily recognized in our samples, doubtless this type is characteristic of the condition. In a single instance distinctly visible floccules of mucus were obtained from the sieve. The sieve residue from many samples of milk revealed minute, irregular, flattened particles. These on examination proved to be small sheets of flattened, squamous, epithelial cells. They are evidently exfoliated particles from the lining of the lower teat canal. Examination of scrapings from the teat canal below the sphincter revealed cells of similar character and arrangement. They may be disregarded as having pathological significance since they evidently result from mechanical pressure during milking. Evidently they are too small to be seen during the routine examination of fore milk, or they strike the sieve with such force as to be broken and pass through.

It is obvious that the condition we have described is an udder inflammation of a mild character. The organisms encountered are capable of producing a severe mastitis and are similar to those noted previously by one of us (1). It is of further significance that 5 of the cows developed clinical mastitis at varying periods after our examination. In these instances the organism present during the mild attack, characterized by the appearance of a few flakes, was likewise overwhelmingly predominant in the severe clinical condition. However, it may be said that although a sufficiently pathogenic organism was present, its numbers were too small to be of much significance. A single case may be cited to show that considerable variation in the number of organisms occurs from time to time even though the milk is drawn at the same hour and by the same individual each day.

Case 1429. January 10, 1927. Left fore quarter. Milk contained characteristic floccules. There were 540 non-hemolytic streptococci per cubic centimeter. There was considerable sediment composed of leucocytes. The milk from the other three quarters was normal and contained 260 organisms per cubic centimeter.

January 11. Flocculi were present in the milk from the left fore quarter. There was an excessive amount of sediment composed of leucocytes. The streptococci numbered 7640 per cubic centimeter. The milk from the other three quarters was normal, and although examined daily never showed abnormalities nor contained streptococci throughout our observations.

January 12. Floccules of leucocytes were present in the milk from the left fore quarter. Leucocytes were found in large numbers in the sediment. A total count of 260 bacteria per cubic centimeter was obtained; 30 per cent were streptococci.

January 13. No flakes were observed in the milk from the left fore quarter; there was, however, the usual excessive amount of sediment. The count rose to 5120 non-hemolytic streptococci per cubic centimeter.

January 14. No floccules were present, but 5900 streptococci per cubic centimeter were found.

January 15. Floccules were found and the count fell to 600 streptococci per cubic centimeter.

January 16. No floccules; 5640 streptococci per cubic centimeter.

January 17. Floccules; 2040 non-hemolytic streptococci per cubic centimeter.

January 18. No floccules; 480 non-hemolytic streptococci per cubic centimeter.

Other cases similar in character have been studied with much the same result. There apparently exists no correlation between the actual number of streptococci and the presence of the floccules. The protocol further brings out the point previously mentioned that the number of organisms may be so scarce at certain times that they may be overlooked.

It might also be suggested that there existed within the udder an organism capable of inciting a mild inflammation which could not be cultivated by the methods outlined. To test this possibility, we inoculated 13 guinea pigs, averaging 250 grams in weight, with all the sediment obtained from centrifuging 25 to 50 cc. of milk drawn directly from the involved quarter. The animals were under close observation for ten days following inoculation and as far as we could tell remained well. Thirty five to forty days later they were killed and agar slants inoculated with bits of spleen. In no instance was B. abortus or other suggestive organism isolated. In addition the milk from all involved quarters failed to agglutinate B. abortus in sufficiently high titer to suggest severe udder infection with this organism. As a further confirmation, films from all sediments were fixed and stained. As a rule the bacteria encountered on microscopic examination could be cultivated. In a number of instances phagocytosis of streptococci and staphylococci was encountered and affords presumptive evidence that such organisms were producing sufficient irritation to arouse the defensive mechanism.

DISCUSSION

The microscopic and bacteriological findings are suggestive that the irregular appearance of elongated, tenacious particles in the milk is associated with bacterial inflammation. The organisms encountered are similar to those usually met with in bovine mastitis. In a number of instances palpaple lesions, usually consisting of irregular, ill defined, firm areas in the ventral portion of the quarter or in the region of the cistern and large ducts, suggested a chronic inflammation. This is well borne out in many instances by the number of attacks extending through one or more lactation periods. However, in the majority of instances lesions are not apparent. The excessive number of leucocytes and the increase in the titer of the blood serum proteins in the milk are indicative that inflammation exists. The castlike character of the floccules argues that certain small lactiferous tubules are involved. The irregular appearance of the floccules in the milk is suggestive that under certain conditions leucocytes are bound together by mucus and may represent casts of some of the small ducts; due to mechanical or other causes they are released and finally reach the cistern. The readiness with which they are detected depends entirely on their size. The tenacious character of the milk sediment in cases where no visible flocculi occur, suggests that the aggregates of leucocytes and mucus are too small to be detected with the unaided eye.

In addition to these points, samples of relatively fat-free milk from 14 affected quarters were tested with cow serum precipitin by the method described by one of us (2). In every instance the milk reacted well above the level of normal milk, showing that sufficient inflammation existed to produce the increased elimination of blood serum proteins.

From the history and our findings in many instances, it is apparent that these cows are relatively resistant. The organisms as a rule are held pretty well in check; if this were not the case the whole udder would soon become involved. In a number of cases this resistance was in some way overcome and in consequence the quarter became clinically involved. Occasionally a quarter may become infected, flakes appear in the milk during a few milkings, and the organism disappear from the quarter. This would explain the case in which no pathogen was found.

Of our series of cases it may be said that we were dealing with several well defined degrees of resistance. Those animals which have one attack and recover must be regarded as most resistant; those which have several attacks and show no definite lesions are perhaps less resistant, the third group which have had several attacks and whose udders show certain abnormalities are on the whole still less resistant; and finally the group whose resistance was overcome by the infecting agent afford a further example of at least a temporary condition in which the resistance was largely overcome. It must be recognized that such cases as we have described are as dangerous from the standpoint of spreading udder infection as severe clinical cases.

It may be said that the organisms which we have encountered are not regarded as pathogenic for humans. However, it is possible that organisms from human sources gaining access to the udder may set up a mild inflammation not essentially different from the type encountered. With this in view we regard the usual method of withholding the milk of such cows from the general supply as essential.

SUMMARY

A condition characterized by the irregular appearance of tiny, elongated, tenacious floccules in the milk of certain cows is described. In the majority of instances the involved quarter presents little that is abnormal; in certain cases firm areas about the cistern and milk ducts were noted. The floccules characteristic of the condition are composed of masses of leucocytes and mucus. Organisms of the same types (non-hemolytic and hemolytic streptococci and hemolytic staphylococci) as those found in mastitis are present in the "flaky" milk. An increased number of leucocytes and a relatively high blood protein elimination are characteristic. The findings suggest a mild but prolonged mastitis.

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CAMEMBERT CHEESE FROM PASTEURIZED MILK*

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INTRODUCTION

The quality and uniformity of Camembert cheese is determined to a great extent by the quality of the milk used from day to day. Pasteurizing the milk has been shown to improve the quality of Cheddar cheese. Since faulty flavors and textures in Cheddar and Camembert cheese are probably caused by similar microörganisms, it seems very possible that pasteurization of the milk can also be successfully applied in the manufacture of Camembert cheese.

Previous attempts to make cheese from pasteurized milk

Numerous investigators have attempted to apply pasteurization as a means of reducing or eliminating the daily changes in the process of cheesemaking, which are largely due to variation in the biological properties of the milk.

Dunne (1) in 1918 reported that in 1908 and 1909 a large number of dairies in Denmark produced hard cheese from pasteurized milk for the chief purpose of ridding the milk of pathogenic bacteria. O. Wennevold was the promoter of the idea of pasteurizing the milk for cheesemaking in Denmark. In 1915 he stated that pasteurizing temperatures of 149° to 158°F. were becoming general and gave good results.

Atkinson (2) in 1922 found that a better cheese was obtained by the "holder" than by the "flash" method. Practically no difference was observed in the time of coagulation when the milk was pasteurized at 145°F. for thirty minutes and a slightly larger amount of starter added. The flavor of the cheese was superior and the yield greater.

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Murray (3) in 1923 obtained similar results.

Gow (4) in 1922 reported that in South America Gouda cheese was manufactured successfully from milk pasteurized by the holder method.

Stevenson (5) in 1923 stated that the manufacture of cheese from milk heated to a temperature of 160° to 165°F. for an instant, cooled, and set with rennet, without the addition of acids or salts, was common in New Zealand. The advantages in favor of pasteurized milk cheese were: Improved flavor, greater uniformity, larger yield, better keeping quality, and simplified process of manufacture.

Price (6) in 1927 partially reviews investigations previous to the year 1922. In his own experiments he showed that milk pasteurized at 145°F. for 30 minutes produced a better quality of cheese than any other holder methods of pasteurization tried.

Kneuttinger (7) in 1926 found that pasteurization of the milk produced inferior Tilsit cheese. He stated that a bitter flavor resulted, perhaps from the influence of the heat upon the albuminoids and salts of the milk, or the increased retention of whey in the curd.

In 1926 Marquardt and Hucker (8) reported that cheese made from pasteurized milk by the holder method showed the greatest improvement when the milk was produced under satisfactory sanitary conditions.

Previous attempts to make Camembert cheese from pasteurized milk

Little has been done to find out whether pasteurized milk can be made successfully into Camembert cheese.

Dunne (1) previously mentioned, reported in 1918 that during the war Denmark produced good Camembert cheese from pasteurized milk to supply Scandinavia.

Golding (9) in 1913 made cheese from pasteurized milk in attempts to improve the keeping quality and to prevent gassy fermentation. Pasteurization temperatures of 150°F. were used. The quality was found to be decidedly improved and the yield slightly greater.

Selection of pasteurizing method

It has been shown that pathogenic and other undesirable organisms commonly found in milk are destroyed or inactivated by exposure to a temperature of 145°F. for thirty minutes. Practical experience has also proved that satisfactory Cheddar cheese can be produced from milk pasteurized by the holder method. This method does not injure the coagulability of the milk, since a good coagulation following this treatment can be obtained without the addition of chemicals.

These facts led to the use of the holder method of pasteurizing in the following experiments.

PURPOSE AND PROCEDURE OF EXPERIMENTS

Purpose of experiments

There were 25 lots of cheese made. Lots 1 to 15 were intended to determine the correct conditions for curdmaking. Based upon their results lots 16 to 25 were planned to determine the effect of the pasteurization upon the quality of the cheese.

Plan of procedure

The milk which was used in the 25 paired experiments originated from different sources and consisted of various grades. In each of the lots 1 to 15, 80 pounds of milk was equally divided and placed in two vats. Each vat of milk was made into 10 cheese. In lots 16 to 25 the amount of milk used was increased to 120 pounds and 15 cheese made out of each vat of milk. Four pounds of milk were required for each individual cheese which weighed approximately ½ pound. In each experiment one of the two vats of milk was pasteurized at 145°F. for thirty minutes and cooled to setting temperature. Enough extra milk was added to each vat to make two rennet tests before and after the addition of the commercial lactic acid starter. Previous to renneting the milk was inoculated with a pure culture of Penicillium camemberti, grown on sterilized crackers. Each vat of milk was made into the best cheese possible. The cheese were cured in the manner recommended by Thom and Fisk, Matheson, and Sammis (10, 11, 12).

Testing

The samples of milk, whey, green and ripened cheese were analyzed for fat and total solids by the Mojonnier method and apparatus.

Scoring of cheese

The scoring of the cheese was done by seven members of the staff of the Department of Dairy Industry. The identity of the cheese was unknown to them. Since there was no standard Camembert cheese score, the following score card was selected as a means of determining the quality of the cheese. Perfect score for: Flavor 50, body and texture 30, mold growth 10, color 5, finish 5, total 100.

To determine the quality of each lot of cheese three raw and three pasteurized milk cheese were selected and presented to the judges. The average of the scores for the three raw and the three pasteurized milk cheese from each lot was taken to indicate the actual quality of the cheese.

Interpretation of results

"Student's" (13) method of interpreting the data of the paired experiments was used. By this method the odds that a given difference is significant are stated as 30 to 1 or 500 to 1, and so on. Odds of 30 to 1 in this work are held to indicate a significant difference.

Student's (13) tables were modified by Love (14). Love's tables were used in this work.

EXPERIMENTS

Effect of pasteurizing the milk on the score of the cheese

These data were obtained from 23 paired experiments. The differences between the average scores of the two types of cheese are summarized in table 1, which shows also the probable error and the coefficient of variation of the scores. From this table it seems evident that a higher scoring cheese can be produced from pasteurized milk than from identical raw milk. The coefficient of variation tends to show that the quality of the pasteurized milk cheese is more uniform.

In lots 1 to 15 the difference between the scores of the paired observations is 4.82 points, and in lots 16 to 25 3.88 points. The odds that the differences are significant are 197:1 and 100:1 respectively.

The pasteurized milk cheese was inferior in quality to the raw milk cheese in only three out of the 23 experiments. In these instances accidental contamination of the pasteurized milk cheese was responsible for their inferior quality.

TABLE 1

Quality of the cheese made from raw and pasteurized milk

LOT NUMBER	NUMBER OF OBSERVATIONS	TREATMENT	MEAN SCORE	COEFFICIENT OF VARIATION
1-15	13	Raw*	81.48 ± 1.62	10.2
		Pasteurized*	86.30 ± 0.89	5.3
16-25	10	Raw*	78.13 ± 1.11	6.3
		Pasteurized*	82.01 ± 0.67	3.6

* All cheese made from the same milk received the same lot number with the differentiation of "raw" and "pasteurized."

Influence of the quality of milk on the score of the pasteurized milk cheese

In comparing the scores of the raw and pasteurized milk cheese, it was noticed that the difference seemed to be greatest when the raw milk cheese was of inferior quality.

The coefficient of correlation between the raw milk cheese scores and the gain in score of the pasteurized milk cheese in lots 1 to 25 was found to be -0.782 ± 0.054. This indicates that with increasing score of the raw milk cheese, the difference in score between it and the pasteurized milk cheese becomes smaller.

Further it was noticed that with decreasing score of the raw milk cheese the score of the pasteurized milk cheese also decreases. To verify this observation the coefficient of correlation was determined and found to be +0.732 ± 0.065.

If it is assumed that the quality of the raw milk cheese indicates in a general way the quality of the milk from which it was made, it seems logical to conclude from these observations that pasteurization of the milk is most effective when the raw milk is of inferior quality.

Effect of starter on the quality of the curd

The firmness and the acid development of the curd are the factors which determine to a large extent the proper draining and ripening of Camembert cheese. In making cheese from raw milk it is desirable to increase the acidity of the milk by adding starter to such an extent that the proper acid development and firmness of the curd for cutting is reached in one an one-quarter to one an three-quarters hours after setting the milk.

The appearance of whey drops on the surface of the curd is the generally accepted sign that the curd is ready to be dipped into the hoops. In these experiments it was found that this condition was obtained in the average quality raw milk with the addition of 3 to 4 per cent of clean-flavored, sixteen-hour-old starter. Due to the fact that in the milk pasteurized at a temperature of 145°F. for thirty minutes, most of the lactic acid producing organisms are destroyed, it is evident that a larger amount of starter is required. In the course of the experiments it was noticed that 4 to 5 per cent of starter brought about the desired conditions of the curd made from pasteurized milk.

Influence of rennet on the quality of the curd

Thom and Fisk, Sammis, and Matheson (10, 11, 12) recommend 3 to 5 ounces of rennet per 1000 pounds of milk. In these experiments it was found that 4 ounces of rennet gave a good coagulation of the milk and the proper firmness of the curd. To determine whether a greater amount of rennet would be beneficial in making Camembert cheese from pasteurized milk, three experiments were made with increased amounts of rennet. In all 3 cases the effect of the greater amount of rennet on the quality of the curd was unsatisfactory. The curd had the tendency to be too firm, and the expulsion of the whey was slow. Very little or no sweating of the curd at the time of dipping, with slow draining of the cheese in the hoops, was noticed.

Effect of pasteurization of the milk upon the yield of Camembert cheese

All the cheese made were weighed every day, from the first day after making until they were wrapped in tinfoil and boxed. The final weight was taken immediately before scoring and disposing of the cheese. Scales graduated to 0.02 pound were used. The yield of green cheese was calculated on the basis of 100 pounds of milk and starter after draining from twenty to twenty-four hours. The yield of ripened cheese was determined in the same manner at the time of scoring.

TABLE 2

Average amount of cheese from 100 pounds of milk and starter

LOT NUMBER	TREATMENT	GREEN CHEESE	RIPENED CHEESE
		pounds	*pounds*
1–13	Raw	21.45	14.02
	Pasteurized	22.25	14.17
16–25	Raw	19.38	13.47
	Pasteurized	19.25	13.41

Table 2 is a summary of the observations.

The experiments show that the average yield for raw and pasteurized milk cheese is practically the same.

Effect of pasteurization on the shrinkage of the cheese

The amount of weight lost by the cheese during the process of curing is influenced largely by the loss of moisture, which depends chiefly on the moisture content of the cheese, the temperature and humidity of the curing room, the length of time of curing of the cheese, and the rapidity and intensity of the mold growth previous to wrapping and boxing.

These factors were constant for each pair of cheese, but some variations occurred in some respects in the curing of different pairs. A record was kept of the loss of weight for lots 1 to 25, and the percent of shrinkage was calculated.

The loss of weight of the pasteurized milk cheese is greater in lots 1 to 15. This is explained by the fact that in lots 1 to 8 the same amount of starter was used in each pair of the raw and pasteurized milk cheese. At the time of dipping and draining the pasteurized milk cheese in each lot had a tendency to retain more moisture than the raw milk cheese, due to the slower development of acid.

In lots 16 to 25, where the amount of starter added to the pasteurized milk is properly adjusted, the loss of weight is practically the same in both types of cheese.

TABLE 3

Loss of weight of raw and pasteurized milk cheese

LOT NUMBER	TREATMENT	PER CENT OF WEIGHT OF GREEN CHEESE LOST IN CURING
1–15	Raw	34.52
	Pasteurized	36 31
16–25	Raw	29 86
	Pasteurized	30 05

Effect of pasteurization on the fat, total solids, and solids not fat content of the cheese

The green cheese made from pasteurized milk contains more moisture in lots 1 to 15. This accounts for the lower per cent of fat in the cheese. In lots 16 to 25 the composition of both types of cheese is practically the same. The data tend to show that with the proper method of curdmaking it is possible to obtain the same composition in the pasteurized milk cheese that is desired in the raw milk cheese. The percentage of fat, total solids, and solids not fat increases during the ripening process due to the loss of moisture.

To determine whether more fat, total solids, and solids not fat are retained in the cheese made from pasteurized milk, the pounds of fat, total solids, and solids not fat are calculated for the cheese obtained from 100 pounds of milk and starter and summarized in table 5.

The amounts of fat, total solids, and solids not fat retained in the cheese made from pasteurized milk are slightly greater only in lots 1 to 15. The differences are not large enough, however, to be significant.

TABLE 4

Fat, total solids, and solids not fat tests of green and ripened cheese made from raw and pasteurized milk

LOT NUMBER	TREATMENT	FAT		TOTAL SOLIDS		SOLIDS NOT FAT	
		Green	Ripened	Green	Ripened	Green	Ripened
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1–15	Raw	18.48	25.59	34.49	46.09	16.01	20.50
	Pasteurized	18.34	25.63	34.10	46.50	15.76	20.87
16–25	Raw	19.12	26.40	36.32	49.06	17.20	22.60
	Pasteurized	19.19	26.84	36.49	49.63	17.30	22.79

TABLE 5

Distribution of fat, total solids, and solids not fat in raw and pasteurized milk cheese, made from 100 pounds of milk and starter

LOT NUMBER	TREATMENT	FAT		TOTAL SOLIDS		SOLIDS NOT FAT	
		Green	Ripened	Green	Ripened	Green	Ripened
		pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1–15	Raw	3.95	3.60	7.36	6.47	3.44	3.34
	Pasteurized	4.01	3.62	7.49	6.58	3.48	3.33
16–25	Raw	3.69	3.56	7.00	6.60	2.87	2.98
	Pasteurized	3.69	3.58	7.00	6.59	2.92	2.97

Effect of pasteurization upon the keeping quality of the cheese

After scoring, some representative samples of each lot of cheese were kept at a temperature of 53°F. for one to four weeks, to determine whether there existed any difference in the keeping quality of the cheese.

The results did not lead to any definite conclusion. The keeping quality of the cheese is determined largely by the quality of the milk, the moisture content of the cheese, and the mold growth. Where these three factors were nearly the same, practically no difference in the keeping quality of the cheese could be observed. However, it was noticed in the course of the experiments that faulty flavors apparent at the time of scoring were more pronounced when the cheese were held for two to four weeks. Bitter flavor especially seemed to increase with the age of the cheese.

TABLE 6

Fat, total solids, and solids not fat lost in the whey

LOT NUMBER	TREATMENT	FAT	TOTAL SOLIDS	SOLIDS NOT FAT
		per cent	*per cent*	*per cent*
1–15	Raw	0.169	0.659	0.490
	Pasteurized	0.116	0.654	0.537
16–25	Raw	0.089	0.657	0.568
	Pasteurized	0.071	0.668	0.597

Effect of pasteurization upon the fat, total solids, and solids not fat content of the whey

Most of the whey drippings were collected at the time of dipping the curd. After the cheese had drained for one and one-half to 2 hours, representative samples of the whey collected from the raw and pasteurized milk were analyzed.

The results in lots 1 to 25 show that the average per cent fat lost in the whey from raw milk is greater than in the whey from pasteurized milk. The mean difference in lots 1 to 15 is 0.053 per cent, and in lots 16 to 25 0.018 per cent. The odds that the differences are significant are 1666:1 and 1999:1 respectively. The total solids content in the whey from both types of cheese in lots 1 to 25 is so variable, that the differences +0.005 for lots 1 to 15 and −0.011 for lots 16 to 25 are insignificant, since the odds are only 5.27:1 and 5.52:1 respectively. The calculated loss of solids not fat is greater in the whey from pasteurized milk. The mean differences are 0.047 for the lots 1 to 15 and 0.028 for lots 16 to 25. The odds that these differences are significant are 2247:1 and 1666:1 respectively.

CONCLUSIONS

1. Camembert cheese can be successfully made from milk pasteurized at a temperature of 145°F. for thirty minutes.
2. Pasteurized milk produces a cheese of better average quality and uniformity.
3. The improvement of the quality of cheese is greatest when the raw milk previous to pasteurizing is of inferior quality. The quality of the pasteurized milk cheese improves with improving quality of the raw milk.
4. In making Camembert cheese from pasteurized milk a larger amount of starter should be used.
5. Pasteurization has practically no influence upon the yield of ripened Camembert cheese.

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BACTERIOLOGICAL METHODS OF EXAMINING ICE CREAM*

FOREWORD

The American Dairy Science Association, through its committee on bacteriological methods, is contemplating the formulation of a complete set of bacteriological procedures useful in controlling the quality of all dairy products. This committee expects to act largely through sub-committees appointed from men in the organization who have had experience with the bacteriological analysis of various dairy products. Obviously, the formulation of such methods should not be left to the arbitrary decision of a committee, but should be the result of suggestions and criticisms coming from all interested parties, whether or not they are members of the American Dairy Science Association. The committee, therefore, wishes to serve as a center about which methods satisfactory to the largest number may be evolved.

The purpose of this preliminary report is to submit to the ice cream industry at large, proposed methods of sanitary control of ice cream. These will be revised later in accordance with the suggestions received and the judgment of the committee, before they are finally adopted by the American Dairy Science Association and included in the report on bacteriological methods of analyzing dairy products.

AGAR PLATE METHOD OF ICE CREAM ANALYSIS

Preparation and sterilization of equipment

Glassware. All glassware should be sterilized in the hot air sterilizer at 170° to 180°C. for from one to two hours. Pipettes and Petri dishes should be wrapped in paper[1] or enclosed in metal containers to prevent contamination upon removal from the

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[1] Imported Kraft wrapping paper withstands sterilizing temperatures excellently without charring.

sterilizer. In arranging the material in the hot air sterilizer, care should be taken to permit free circulation of air about each package or piece of glassware.

Method of sampling

Liquids. Cream, milk, skim milk or ice cream mix should be sampled by means of a sterile sampling tube. Such a tube can be made by flaming the ends of a 36-inch piece of ½-inch glass tubing. Aluminum or other metal tubes may also be used. One to four tubes may be completely wrapped in paper or the tubes may be enclosed in a metal case before sterilization. If the cream or milk to be sampled is in cans, a composite sample may be obtained by agitating, and then slowly inserting the tube to the bottom of each can, removing, thereby, a sector of the contents and placing in a sterile sample bottle. The sample bottle best adapted to this work is a wide mouth, ground glass stoppered bottle of about 120-cc. capacity. Fifty to 100 cc. of sample should be taken in each instance.

If the liquid to be sampled is in a vat, the contents should be agitated thoroughly, then sampled by means of the sterile sampling tube in at least six places (preferably more) in the vat.

Liquid samples taken from the spout of the homogenizer or freezer should be obtained by quickly passing the bottle under the opening at periodic intervals during the discharge so as to get representative samples of the material delivered from the machine.

Solids. Sugar, gelatin, and skim milk powder should be thoroughly mixed with a sterile spatula and a portion placed in a sterile sample bottle.

Frozen ice cream should be sampled with a sterile butter trier, after removing the surface inch from the point to be sampled. The core thus removed should be cut into short pieces with a sterile spoon or spatula and placed in a sterile bottle.

Care of the samples

All samples should be placed in cracked ice immediately and carried directly to the laboratory as quickly as possible.

Measuring the sample

The sample of ice cream may be either weighed or measured. With ice cream it is frequently difficult to expel all the air, with the result that one 10 cc. volume may contain more or less ice cream than another. Variations in the viscosity of the mix and in the cleanliness of the pipette may cause more or less of the mix to adhere to the glass. The gravimetric method has the disadvantage of being more tedious and more time-consuming, but, in general, gives more accurate and less variable results. The preference for the gravimetric or volumetric method will depend on the nature of the analytical work. For ordinary plant control work, where routine check is being made on the quality of the ingredients and the final product, the volumetric method is sufficiently accurate. On the other hand, for research and public health work, where accuracy and comparability of results are essential, the gravimetric method should be used. However, if consecutive samples are being taken at the various stages in the process of ice cream manufacture, all determinations should be made on the same basis, either gravimetric or volumetric. The former method gives results showing the estimated numbers of colonies developing on agar plates per gram, the latter, the number of colonies per cubic centimeter.

If the sample to be analyzed is frozen ice cream, it should be melted and the air expelled by heating in a water bath at 45°C. (113°F.) for fifteen minutes. If a higher temperature is used for melting the ice cream, it is likely to injure the organisms, and lower temperature is likely to necessitate a sufficiently long exposure to permit noticeable growth of the bacteria. When the volumetric method is used for ice cream, extreme care should be taken to insure complete expulsion of the air, and to rinse the pipette several times in the dilution water until the pipette is reasonably clean and free from ice cream.

Volumetric method. Liquids. The larger the volume of sample used in making the first dilution the less will be the per cent of error in measuring the sample. This is especially true of liquids of high viscosity such as heavy cream and melted ice cream mix.

The amounts most commonly used are: 1 cc. of sample in 99 cc. of sterile water, 5 cc. of sample in 95 cc. of sterile water, or 10 cc. of sample in 90 cc. of sterile water, giving dilutions of 1:100, 1:20, and 1:10 respectively. For all volumetric samples, the volume of the sample plus the volume of the sterile dilution water should equal 100 cc. The dilution, therefore, should be expressed as in "1:10," "1:100," etc., and the results in terms of "plate count per cubic centimeter—50,000, etc." Not more than two significant left-hand digits should be used in reporting numbers.

Gravimetric method. Solids. The results of gravimetric analyses are reported in terms of "plate count per gram—50,000 etc." Analyses of gelatin, sugar and skim milk powder, are usually made on a gravimetric basis, by weighing ten grams on sterile paper and placing in 90 cc. of sterile water under aseptic conditions. From this 1:10 dilution, appropriate dilutions are made and plated in the usual manner. In the analysis of gelatin it is necessary to warm the dilution blanks to insure equal distribution of the sample.

Fruits, such as strawberries in heavy syrup, may be weighed on sterilized paper conterpoised on the balances. Place two sheets of sterile glazed filter paper on the pans of the balance and weigh a 10 gram sample. Under aseptic conditions, introduce the fruit, together with the single thickness of filter paper, into the dilution bottle. In this manner the contamination of the paper from the scale pan is prevented.

Gravimetric method. Liquids. If a 10-gram sample of cream, melted ice cream, condensed milk, etc., is weighed directly into a dilution blank, it does not matter how much adheres to the pipette or how much air remains in the melted ice cream.

Method of weighing sample directly into dilution blank. A dilution blank containing 90 cc. of sterile water, and a pair of Cornet forceps are counter-balanced on a good grade of torsion balances. It is more convenient to have a beaker of water on each pan of the balance to facilitate counter-balancing. The instrument should be sufficiently sensitive so that one drop of water will disturb the balance. After counter-balancing to within one drop of the true balance, add a 10-gram weight to the pan opposite the dilution blank; remove the stopper from the dilution blank, taking care that it does not touch anything. Flame the mouth of the flask and, by means of a pipette introduce the sample to be analyzed until the 10-gram weight is accurately balanced. Flame the mouth of the dilution bottle and replace the stopper. Test the accuracy of the weighing by dropping one drop of the sample in the beaker on the opposite pan, if it is close enough so that one drop throws the balance to the other side, it may be considered sufficiently accurate for use in the analysis. If cotton stoppers are used in the dilution blanks, the Cornet forceps are used to suspend the stopper over the edge of the pan so that it is not contaminated by touching the pan. If glass- or rubber-stoppered dilution bottles are used, the Cornet forceps are unnecessary.

PREPARATION OF AGAR PLATES

Media. For ice cream analysis, several media have been found satisfactory, and others not mentioned here, no doubt, could be used with equal satisfaction. Most public health laboratories, however, use the standard agar as described in detail in the fifth edition of Standard Methods of Milk Analysis, published by the American Public Health Association, 370 Seventh Avenue, New York City. Briefly, this media is prepared as follows: Add 0.3 per cent Bacto-beef-extract (or other brands giving comparable results), 0.5 per cent Bacto-peptone (or other brands giving comparable results), and 1.5 per cent market agar to the desired number of liters of distilled water; dissolve in an autoclave for from forty to ninety minutes after the pressure has reached 15 pounds, the length of time depending on the quantity of media being prepared. Remove the media and determine its reaction. If necessary, standardize the reaction to pH 6.6. This may be done according to the method described in the Standard Methods of Milk Analysis. After standardization, the media should be filtered through cotton or Buchner funnels. Place 10 cc. of media in test tubes or 55 cc. in flasks and sterilize at 15 pounds pressure for twenty minutes or by heating in flowing steam on three successive days for twenty minutes each day.

Milk powder agar, as described by Ayers and Mudge, has been found very advantageous in the determination of different types of bacteria in ice cream. The following specific directions for making milk powder agar are quoted from the original article by Ayers and Mudge.[2]

Detailed directions for preparation of 1 liter of milk powder agar

Medium A ingredients for 1 liter:

(a) 5 grams skim milk powder
1 gram sodium dibasic phosphate (Sorensen's phosphate) ($Na_2HPO_4-2H_2O$)
In 250 cc. distilled water

(b) 5 grams peptone
3 grams extract
In 250 cc. distilled water

Mix (a) and (b) and add 500 cc. of double strength (3 per cent) washed-agar solution.

The detailed directions must be followed accurately if satisfactory and constant results are to be obtained. The medium is very easy to prepare when the various steps are understood and the process completed once. It may appear complicated because of the complete details which are given of each step in the process and which make the preparation of the medium appear somewhat long.

To make the milk-powder solution "a" use a good grade of skim-milk powder made by the spray process, and prepare the following solutions:

(1) 5 grams milk powder
20 cc. distilled water

(2) 1 gram sodium dibasic phosphate (Sorensen's phosphate)
5 cc. distilled water

(When making more than 1 liter, the same proportions of milk powder to water and phosphate to water must be used; therefore, to make 5 liters multiply each amount by 5.)

Weigh out 5 grams of skim-milk powder and pour on to 20 cc. of cold distilled water in a small beaker. Stir until thoroughly dissolved. In another beaker, dissolve 1 gram of sodium dibasic phosphate (Sörensen's phosphate) in 5 cc. of distilled water. Warm to dissolve phosphate quickly. Sörensen's phosphate, $Na_2HPO_4-2H_2O$, must be used.

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[2] Ayers, S. H., and C. S. Mudge. Milk powder agar for the determination of bacteria in milk. Jour. Bacteriol., 5: 565–588 (1920).

Add the phosphate solution (2) to the milk powder (1). Place the beaker, containing (1) and (2) mixed, in a water bath with water at about 30°C. and heat the milk powder phosphate solution to about 60°C. This should take about ten minutes. At temperatures between 50° and 60°C. a flocculent grayish precipitate will appear. Continue the heating until the precipitate appears, then steam in an Arnold or other steamer for five minutes, or until the precipitate appears white. Then dilute the milk-powder solution about one-third with distilled water and steam five minutes longer. Too long steaming will cause the solution to turn dark and should be avoided. The whole heating period should not be more than twenty or twenty-five minutes.

Decant the solution while hot on to a filter paper, taking care to keep the precipitate in the beaker until most of the liquid is through. Then pour the precipitate on the filter and wash with a little distilled water. If the milk-powder solution has been properly heated it will filter readily, provided the filter paper is not too hard. "J. Green" Grade 588 and "Ilmenau" filter paper have given good results.

The filtrate, which is of a yellowish-white color, will appear cloudy and can not be filtered clear in the concentration used. This makes no difference, because it is clear in the dilution of the final medium.

Make up the filtered milk-powder solution to 250 cc. with distilled water. This completes the milk-powder solution "a."

To make the peptone-extract solution "b" dissolve 5 grams peptone and 3 grams Liebig extract in 100 cc. distilled water by steaming in the Arnold sterilizer or by boiling over flame for twenty minutes. Filter until clear and make solution up to 250 cc. with distilled water. This completes solution "b." "Difco" peptone has been used in our experiments because of its hydrogen-ion concentration, which is near the neutral point, and because, with the extract in the proportion of 5 grams peptone to 3 grams extract, a precipitate is usually formed which permits filtration with a resulting clear solution. For a standard medium, whatever makes of peptone and extract are selected should be universally used.

The milk-powder solution "a" is now mixed with the peptone extract solution "b" which gives a total volume of 500 cc. To this mixture 500 cc. of double strength (3 per cent) washed-agar solution is added. This completes the medium, which is now ready for sterilization.

We specifically mention distilled water. Tap water may or may not contain dissolved substances, the effect of which on media making and bacterial growth is unknown. By the use of distilled water this uncertainty is obviated and one is assured of a constant definite solvent, "standard" everywhere.

A stock solution of double strength (3 per cent) washed agar is prepared and put up in flasks and sterilized. This agar is then ready at any time for use. The agar should be put up in flasks in amounts suitable for the amount of medium to be made at any one time. It is not desirable to use part of a flask of agar, then resterilize and hold for future use. Repeated heating lowers its jelly strength. The flasks should be stoppered to prevent evaporation. To prepare a liter of 3 per cent washed agar, weigh out 30 grams of agar and place in a flask with 200 cc. of distilled water. This proportion should always be used. Allow it to stand for twenty-four hours, at room temperature, with occasional shaking. Then pour off as much water as possible, using a piece of cheesecloth over the top of the flask, and add distilled water enough to make up again the original volume.

Allow the agar to stand another twenty-four hours, and then pour off the agar on to a cotton flannel cloth in a funnel and wash once with a liter of distilled water. Let the agar drain and then press out as much water as possible by squeezing the filter cloth with the hands. A container large enough to hold the agar is counterpoised on the laboratory scales, and the agar placed in it. In the opposite pan is placed 30 grams for the agar and 1000 grams for the weight of the water in which the agar is to be dissolved. Then water enough is added to make up this weight. This will make a liter of 3 per cent agar. Dissolve the agar by heating in the Arnold sterilizer, then filter through cotton flannel or absorbent cotton until clear.

Whey agar and casein digest agar have not been so widely used in ice cream analysis. Carbohydrate media such as dextrose agar, lactose agar, etc., may be prepared by adding one per cent of the carbohydrate to the plain agar previously described. The addition of carbohydrate to the plain medium increases the number of colonies appearing on the plates.

If it is desired to check the results of analysis in one laboratory with those obtained in another laboratory, it is essential that the media employed in both laboratories be the same.

Making the agar plates

After placing 1 cc. of the desired dilutions in Petri dishes, 10 cc. of agar should be admitted to each plate under aseptic conditions.

If the agar is in flasks, care should be taken to pour the same amount of agar into each plate. The number of plates poured will depend upon the accuracy desired. Ordinarily, duplicate plates should be prepared from each dilution, although this may not be necessary in routine plant control work.

Incubation

The Standard Methods of Milk Analysis recommends incubation of plates for forty-eight hours at 37°C. A more complete count will be obtained by adopting the plan followed in some laboratories of incubating forty-eight hours at room temperature, followed by forty-eight hours at 37°C.; still other laboratories use one week incubation at room temperature. If results are to be compared with the results of another laboratory, the incubation time and temperature should be the same in both laboratories.

Plates should be inverted during incubation to prevent spreaders. The atmosphere in the incubator should be kept moist so as to prevent the drying up of the media. Care should always be taken not to stack plates in piles of more than three, in order to insure equal incubation of all plates. Plates should not be disturbed during incubation, otherwise the water of condensation will flow across the plate and cause growth on the surface of the agar.

Counting the colonies

A convenient method of counting the colonies is to invert the plate and mark each colony with ink or a wax pencil as it is counted, at the same time keeping count with a tally meter in the other hand. This method greatly increases the accuracy of counting by preventing confusion of the count, due to interruptions or to counting the same colony twice. All plates in a series should be counted at as nearly the same time as possible. After counting the colonies with the naked eye, all plates should be carefully examined with a hand lens ($3\frac{1}{2}\times$) to detect colonies too small to be seen with the naked eye. Occasionally, it may prove necessary to examine the plates under the low power of a compound microscope in order to determine the true nature of apparent colonies.

MICROSCOPIC COUNT OF BACTERIA IN ICE CREAM (FABIAN'S MODIFICATION OF THE BREED METHOD)[3]

In working with ice cream it is often desirable to obtain certain information that cannot be obtained by any other method than that of examining a small amount of ice cream directly under the microscope. While there has not been as much work done with this method for ice cream as has been done with milk, yet the committee feels that there is sufficient merit in the method to incorporate it in this report. The direct microscopic method for ice cream may be used to best advantage with the unpasteurized product and in the raw materials going into the mix. However, it is also of service in detecting the presence of excessive numbers of bacteria, use of moldy fruits and the like in the pasteurized product.

Apparatus required

The apparatus required for this method is the same as that used in the Breed method as applied to milk, excepting an analytical balance. A microscope, microscopic slide, an analytical balance, guide plate (2 by 4½ inches), staining jars, stain, xylol, alcohol, etc., should be available.

Preparation of ice cream film

The ice cream film, to be representative, should be made from a thoroughly mixed sample. Slides used in this work should be exceptionally clean if the best results are to be obtained. The slides are placed on the balance and counterpoised; 0.01 gram of ice cream is then weighed upon each end of the slide as quickly as possible in order to reduce the error due to evaporation. A platinum loop has been found to work very well for putting the ice cream on the slide. The 0.01 gram of ice cream is then spread uniformly over an area of 1 square centimeter, using a clean needle.

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[3] Fabian, F. W. A bacteriological study of the homogenization process in making ice cream. Jour. Dairy Sci., 8: 246–269 (1925).

This area may be conveniently measured by placing the slide upon the guide plate or any surface ruled in square centimeter areas. After the film is spread, the slide should be placed upon a warm surface for drying. The drying should not be too rapid for this may crack the film and cause it to peel from the slide. However, the drying should be completed quickly (within ten minutes) or growth may take place.

After the film has been prepared as above described, the slides are ready for the staining process. They are first dipped in xylol or other suitable fat solvent for five minutes to remove the fat. Longer exposure is necessary for ice cream than for milk, due to the larger amount of fat present. The xylol is then drained off and the slide again placed on a warm surface to evaporate the remainder of the xylol. The slides are next immersed in 70 to 95 per cent grain or denatured alcohol for about twenty minutes. The alcohol is poured off and the slide again placed on a warm surface. The film is then stained thirty seconds with Loeffler's methylene blue, prepared as follows: saturated alcoholic solution of methylene blue, 30 cc., caustic potash (0.01 per cent solution), 100 cc. Care should be taken in selecting the methylene blue. Fresh preparations of the stain from a satisfactory source should be used.

The film is then ready to be decolorized. This may be done by first draining off the excess stain, and placing the slide in 70 to 95 per cent alcohol. The length of time it should remain in the alcohol is a matter of judgment and experience; usually dipping the slide in and out once or twice is sufficient. In case the decolorization is carried too far, the preparation may be re-stained without apparent injury. The film should be warmed again after it has been decolorized; this seems to be necessary for best results.

In routine practice staining jars may be used to good advantage. Place four jars in a row, containing xylol, 95 per cent alcohol, Loeffler's methylene blue, and 95 per cent alcohol, respectively. The preparation of four slides may be started and carried through the whole procedure at the same time, and these followed by four more, thus saving considerable time.

Standardizing the microscope

In order to obtain quantitative data the size of the microscopic field must be known. The only equipment necessary for standardization of the microscopic field is a stage micrometer ruled in hundredths of a millimeter, and a suitable ocular (6.4 × gives approximately the field desired). A mechanical stage is not necessary but very convenient for this work.

The diameter of the microscopic field should be adjusted to 0.205 mm., by focusing the microscope on the graduations of the stage micrometer, and adjusting the draw tube so that the desired diameter is obtained. Each field will then cover an area of $\frac{1}{8028}$ square centimeter ($\frac{1}{8000}$ square centimeter is the approximate figure and the one generally used). When this adjustment is made, then about $\frac{1}{300,000}$ part of a gram of the dried ice cream is visible in one microscopic field. The number of bacteria found in each field, therefore, should be multiplied by 300,000 to give the estimated number of bacteria per gram. Obviously, more than a single field should be counted, and the average number per field used in the calculation. For routine work at least twenty-five representative fields should be counted for each sample of ice cream.

A special ocular micrometer with a circular ruling divided into quadrants should be used for counting ice cream containing few bacteria. With this micrometer, adjust the microscope so that the diameter of the circle on the eye-piece is 0.146 mm. This changes the value of each field examined to $\frac{1}{600,000}$ part of a gram, and the number of bacteria found in each field should be multiplied by 600,000 to give the estimated number of bacteria per gram. This method has the added advantage of examining only the center of the field where definition is good.

Significance of the microscopic count

No bacterial standard, based on this method, has been worked out for ice cream. However, it is felt that the method gives certain data as to the type of bacteria present that can be obtained best in this way.

This method, like all known methods for determining numbers of bacteria, should be regarded as giving an estimate only. Errors due to evaporation during weighing, inaccuracies in weighing such small amounts of ice cream, faulty staining, uneven spreading of the ice cream, counting objects that resemble bacteria, or failing to see bacteria that are present, are all possible sources of error. While these limitations are recognized as important, yet it is felt that they may be sufficiently overcome to make this method a valuable adjunct in determining the quality of unpasteurized ice cream.

The microscopic preparations of ice cream are more pitted than with milk, due to the large per cent of fat present.

Where the ice cream mix has been pasteurized, the presence of large numbers of dead bacteria makes the microscopic count unreliable for determining the quality of ice cream. However, it is especially advantageous for certain types of work, such as studying the breaking up of bacterial clumps after homogenizing or freezing.

METHODS OF CONTROLLING THE SANITARY QUALITY OF ICE CREAM

Methods of sterilization[4] *of the utensils and equipment*

The sterilization of utensils and equipment is just as necessary in an ice cream plant as in a market milk plant, a creamery or a condensery. In general, sterilization should be accomplished with heat rather than with chemicals. After thorough washing, the utensils should be treated with steam or hot water. Drying is necessary if bacterial growth is to be prevented and this may be accomplished with driers or by such thorough heating with steam or hot water that the moisture remaining after careful draining will be evaporated.

All piping should be of the sanitary type and should be taken down and thoroughly washed, steamed or scalded, and dried each time it is used. The vats employed for mixing or holding should be given just as careful attention as the small containers. Homogenizers and similar machines are very difficult to keep in a sanitary condition and may be important sources of contamination unless considerable attention is given to them. These machines should be thoroughly washed so that there will be as much drying as possible. Before using, it may be advisable to run hot water through the machine for several minutes in an attempt to remove or destroy the organisms that may have developed in the machine since it was cleaned. Surface coolers need careful attention and preferably should be steamed or scalded just before using.

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[4] The term sterilization, as used in this case, refers to practical sterility and not absolute sterility.

Freezers present a difficult problem in sterilizing with steam because the low temperature at which they are operated necessitates slow and gradual warming before the steam is admitted. The freezer should either be allowed to stand an hour after washing and before using hot water and steam, or rinsed three or four times with gradually increasing temperatures of the successive rinse waters. If the temperature is gradually increased in this manner, the machine will not be injured by the introduction of steam.

Cans, vats, freezers, etc., which are to be dried by the heat in the metal, should be so left that the water vapor can readily escape. When the drying is complete such equipment may be closed to prevent air contamination, although this source of contamination is not usually serious in a plant where there is considerable moisture about.

The use of chemical disinfectants

Chloramine and hypochlorite disinfectants are frequently used in ice cream plants, especially in the sterilization of freezers. In some plants large volumes of disinfectant are pumped through the entire system. This gives satisfactory results, especially if used in conjunction with steam. Intelligent use of chemical disinfectants necessitates extreme care in the preparation and use of fresh solutions, in order to insure a high available chlorine content.

A home made sodium hypochlorite disinfectant may be made according to the following directions. Add enough warm water to 10 pounds of fresh chloride of lime (bleaching powder) to make a moist paste. After this is done add the remainder of 3 gallons of warm water (not over 120°F). Break up all the lumps in the lime and strain the solution through screen wire. Dissolve 16 pounds of washing soda (sodium carbonate) in 3 gallons of warm water, and add this solution to the chloride of lime solution in a 10-gallon stone jar (do not use metal container). Allow the mixture to stand about twelve hours, stirring two or three times at intervals. Draw off the clear supernatant liquid into gallon earthenware jugs, keeping the liquid as clear as possible and free from the insoluble residue. Discard the residue. The stock disinfectant solution thus obtained will keep a long time if the jugs are tightly stoppered.

For disinfecting freezers, pipe lines, pumps, cans, and other equipment, use a dilution of 1 part stock solution to 100 parts of water. Make a new dilution of the disinfectant each day. It is important that all equipment should be thoroughly cleaned and brushed before the disinfectant is used. Allow the disinfectant to act about three minutes to secure the best results. Where steam is used in addition to the disinfectant, use the disinfectant before using the steam.

Importance of quality of ingredients

The bacteriological condition of certain of the ingredients[5] employed has a direct relationship to the bacterial content of ice cream, both when they are pasteurized and when they are not. In general, the dairy products used in ice cream are the most important sources of bacteria. The quality of the cream and milk used in the mix is a very important consideration in controlling the number of bacteria in the mix.

All dairy products used in the mix should be as fresh as it is possible to obtain them. Both natural and re-constituted cream vary a great deal in bacterial content even after they are heated. Bulk condensed milk may be an important source of organisms, especially when it is held without refrigeration, as may be the case during transportation.

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[5] Olson, N. E., and A. C. Fay. The bacterial content of ice cream. Jour. Dairy Sci., 8: 415–444 (1925).

Gelatin varies a great deal in bacterial content, although there apparently has been a big decrease during recent years in the number of organisms present in this product, due presumably to improved methods of manufacture. In many cases the heating incident to dissolving the gelatin destroys a great majority of the organisms so that the contamination from gelatin may be much less than the original bacterial content of the product would indicate. The small amount of gelatin ordinarily used also tends to make this product responsible for only a small per cent of the organisms in the finished ice cream. In some instances, however, a high bacterial content is accompanied by an objectionable odor and poor gelatinizing properties.

Sugar which is properly protected from dust and dirt ordinarily contains only small numbers of organisms, and is usually insignificant as a source of bacteria in ice cream.

Vanilla extract usually contains only a small number of bacteria, due, in part at least, to the alcohol content.

Importance of plant sanitation

Sanitary conditions are just as desirable in plants in which ice cream is manufactured as in factories handling other food products. Floors, walls, and ceilings which are easily cleaned and impervious to water, an abundance of sunlight, good air conditions, etc., are important considerations when a plant is being built. In the operation of the plant, advantage should be taken of those factors and the plant kept in a clean condition. While proper sterilization of utensils and equipment are undoubtedly more important from the standpoint of the bacterial content of the ice cream than the condition of the plant, a clean, well lighted and well ventilated establishment suggests careful methods to the employees, and establishes confidence among the consumers.

Importance of cleanliness of operators

Cleanliness of the operators is an important factor in the handling of all food products. If the operators are careless in their personal habits, in the cleaning of equipment, etc., an influence on the bacterial content of the ice cream is to be expected. Most diseases of human beings are of human origin, so that personal contact in the manufacture of any food should always be avoided whenever possible. Dirty clothing may be a source from which dust and dirt get into the ice cream, and, moreover, suggests carelessness to both the operator and visitors. The possibility of pathogenic organisms coming from the mouth, nose, hands, etc., makes careful habits among the employees very necessary.

Routine samples which should be taken to discover the cause of high bacterial counts

High bacterial counts from ice cream indicate that something is wrong, either with the quality of the raw materials or with the methods of manufacture, and, accordingly, the immediate cause should be located at once. The bacterial content of the various raw materials used, especially those which are likely to undergo bacterial changes, should be determined. These should be examined in the condition in which they are used and not in the condition in which they are received. The bacterial content of the mix before pasteurization should be determined first, and if this is found to be satisfactory, the analysis of the individual raw material may not be necessary. If the mix is held or aged, counts should be made after storage as well as before. Serious contamination from a piece of equipment can be detected by examining the material before and after it passes through this equipment. With certain machines, for example the homogenizer, the first material through is likely to be much more seriously contaminated than the material going through later. The tendency of such machines as the homogenizer and the freezer to cause an apparent increase in the bacterial content, due to the breaking up of the clumps of bacteria, must be taken into account in studying their influence on the bacterial count.

It is frequently advisable to take samples of the finished product just before it leaves the plant, and also after it has been held in the retailer's cabinet. Alternate thawing and freezing of ice cream, due to inefficient refrigeration should always be avoided.

Interpretative value of bacterial numbers

The total numbers of bacteria in dairy products have been used as an index to the sanitary quality for many years. The use of this index is based on the assumption that the conditions which favor the admission and growth of bacteria are not in keeping with the commonly accepted ideas of the manner in which dairy products should be handled. The factors which cause large numbers of bacteria in ice cream are, poor quality of ingredients, inefficient pasteurization, insanitary equipment, improper aging conditions, and sloven, careless methods of the employees. That is to say, ice cream with a high bacterial content has been neglected in one or more of the above factors. It is possible, however, that ice cream with a low bacterial content may have been made from poor quality ingredients, and that fact masked by pasteurizing at a high temperature. The most serious difficulty with the index value of bacterial numbers is the unavoidable high per cent of error in the count in any method of estimation yet devised. This fact should always be borne in mind in using the results as indexes to insanitary conditions. If used by inspectors or officials the results should only be interpreted as a guide to the plants needing attention and not as an infallible index of insanitary conditions.

To the plant operator, high bacterial counts should indicate that there is something wrong with the methods, or the quality of the ingredients. If detailed inspection of the plant does not reveal to him the cause of high counts, it will be necessary to take routine samples of the manufacturing process to locate the trouble.

High bacterial counts should not be construed by the inspector to mean that the plant is insanitary or that the product is necessarily unsafe, but rather that there is something faulty about the plant methods which can be revealed only by more detailed inspection. On the other hand, ice cream having a low bacterial count should not be regarded as above reproach from a sanitary point of view, due to the possibility of masking the poor quality of ingredients by the use of high temperature or long time of pasteurization. It is always advisable to combine inspection with laboratory findings in order to draw conclusions from the results.

Excessive numbers of bacteria in ice cream are significant largely through the faulty methods which their presence reveals, and not because excessive numbers of bacteria indicate the presence of dangerous disease germs. At best, the bacterial count can be used only as an aid to the inspector and not as a substitute for inspection.

The views expressed in this report are the views of a committee appointed by the American Dairy Science Association. As such they are printed for the general criticism of other members of the A. D. S. A., and any other interested parties.

Separate copies of this report may be secured at cost from the Chairman of the Committee on Bacteriological Methods.

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A COMPARISON OF GUERNSEY SIRES

III. BASED UPON THE AVERAGE PERSISTENCY OF FAT SECRETION DURING THE LACTATION OF THE DAUGHTERS*

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During the past few years, a number of studies have been made of the changes in the rate of secretion of milk and fat during the lactation. It has been found that many factors which may be considered environmental or physiological, influence the shape of the curves of secretion. These factors include season, nutrition, pregnancy, age and frequency of milking. There are undoubtedly many others which fall into this class.

Another important cause of variation in the shape of the lactation curve is due to the inheritance of the animal. Exactly what part of a cow's milk and fat production is due to environmental factors and what part due to inheritance is difficult to determine. The view is held that faulty nutrition and management may lower production but that it is impossible to increase production above the limit set by inheritance(1).

As yearly or lactational milk yield is a very complicated process, it was thought that a step in the analysis of the complicated mechanism of the inheritance of milk and fat secretion might be made by separating the principal parts of the curve of lactational milk secretion and by studying each part separately.

THE SHAPE OF THE CURVE OF MILK SECRETION

a. The rising segment

The first segment of the curve of milk secretion is a period of increasing production. The length of time included varies considerably,—ranging from ten to forty or fifty days. It has been shown (2) that the rising segment of the curve of milk secretion

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may be represented by the equation of a monomolecular chemical change of the form.

$$M = B\left(1 - e^{-k_2 t}\right)$$

in which M is the milk flow at the time t and k_2 is the characteristic constant of decline in the rate of the rise in the curve of milk secretion. As seen in figure 1 the rise in milk secretion becomes less and less as it approaches the maximum and the decline in this rise is exponential. It is assumed that the rise of milk secretion after parturition is due to a limiting chemical reaction.

Recently Gaines and Davidson (3) have suggested that there is formed during pregnancy a hormone which inhibits milk secretion and causes the observed decline in lactation as pregnancy advances. It is further assumed that the production of the inhibitor ceases at parturition and that the amount then present in the circulation is destroyed or eliminated at a rate proportional to its concentration at the moment. This would account for the decline in the increase in milk secretion being exponential.

Data obtained by Asdell (4) indicates that an inhibitory hormone is unnecessary to postulate which defers lactation until the fetus is withdrawn by parturition. It was found by milking pregnant first calf heifers that there was a change in consistency of the secretion obtained and a gradual increase in amount beginning about half way through pregnancy. Pregnancy or changes accompanying pregnancy are therefore, believed to cause the production of a hormone which stimulates mammary tissue growth and milk secretion. (See reference 6 for further information as to the possible sources of this hormone.) In other words, it has been proven experimentally that milk secretion is actually stimulated during the latter part of pregnancy while an inhibitor to lactation has not been demonstrated.

It should not be inferred from this that milk secretion is actually increased during the latter part of pregnancy for it has been clearly shown that there is more rapid decline in milk yield during that period. The observed decline in milk yield of pregnant cows in the presence of a stimulation to mammary development and secretion in immature animals is believed to be caused by the increasingly large requirement of the fetus for nutrients, masking the stimulation observed in the latter part of pregnancy of previously non-lactating cows.

In a late paper Gaines (5) presented a study of 1676 Guernsey records in which k, the persistency constant, was determined for each individual record. It was found that of this number 83 or 5.41 per cent of the lactation curves, the values for k indicated an increasing production for nine or ten months. From this Gaines concludes that some modification of the simple monomolecular interpretation is necessary. He states further that "the group behavior of these ascending records is not in good conformity with the equation type, being somewhat aberrant at the start and finish."

It is hardly to be expected that an equation which is intended to describe only the rate of decline after the time of maximum secretion would describe the ascending curve which, in the cases he describes, continues longer than that normally observed. In other words, he has fitted an equation of the type (2)

$$M_t = M_0 e^{-kt}$$

to data when it clearly calls for an equation of the type (1)

$$M = B\left(1 - e^{-k_2 t}\right)$$

b. The period of maximum production

The rising segment of the milk secretion curve finally blends into a period when secretion is at a maximum for the lactation. This may be a single day, or there may be a number of days during which a maximum flow of milk may be attained. As the yield of milk and fat are calculated by months, it is convenient to consider the month of maximum production in dealing with advanced registry data.

c. The declining segment

After the period of maximum milk secretion, the curve of production declines following an exponential law (7). This law may be stated for our purpose as follows: When all other conditions are uniform, the monthly milk or fat production during the lactation period, after the maximum is passed, is a constant percentage of the preceding month's production. This relation may be expressed mathematically by the equation (2)

$$M_t = M_o e^{-kt}$$

where M_t is the milk produced during any month of the lactation period t. M_o is the initial theoretical production, k the persistency constant and e the base of natural logarithms (8).

It is apparent from the preceding discussion that total yearly or lactation milk yield is dependent upon the numerical value of the constant of decline of the rising segment, the maximum, and the constant of decline after the period of maximum production. Of these, the height of the maximum and the persistency of decline are of greatest importance in their influence on the lactation yield. An exception should be made to those lactations which continue to rise for an extended period (nine or ten months) found by Gaines.

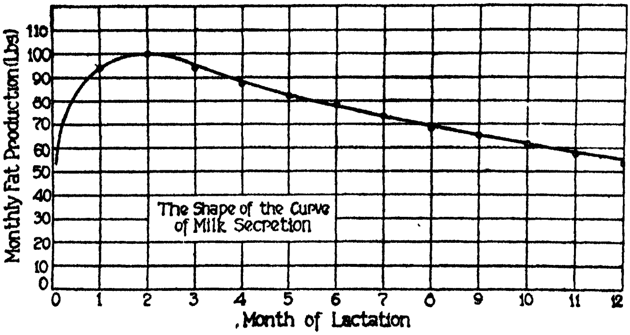

FIG. 1. THE INITIAL RISE AND SUBSEQUENT DECLINE OF BUTTERFAT SECRETION WITH THE ADVANCE OF THE PERIOD OF LACTATION OF GUERNSEY COWS

INFLUENCE OF INHERITANCE ON MILK SECRETION CURVE

With quantitative measures of the chief characteristics of the lactation milk secretion curve, it is possible to study the influence of inheritance with greater precision. In a previous paper data were presented on the variation in maximum monthly fat production of Guernsey Advanced Registry Cows (9). The comparison of the average progeny performance of Guernsey sires with that of the daughter's dams was also given. It is the object of this paper to make a similar study of the persistency of fat secretion during the declining period of the lactation of the same yearly records.

PERSISTENCY OF MILK AND FAT SECRETION

The importance of the characteristic of persistent secretion of milk and fat has not been realized as fully as it should considering its influence upon total yield. In the Holstein Friesian breed, the seven-day and thirty-day tests are still in wide use in the selection of breeding stock although they measure only the maximum production of the cow. The lack of appreciation of this character may be due in part to the fact that there is little in the external conformation of the cow to indicate the presence of this character. Even with a lactation milk record it has been difficult to determine with any exactness the degree of persistency of a cow. Without a quantitative measure of persistency, study of the inheritance of the character has been difficult.

Sanders (10) of Cambridge, in studying the shape of the lactation curve of English milk records, has determined what he calls the *shape figure* (S. F.) of the cow's lactation. The *shape figure* is the ratio of the total lactation yield to the maximum daily yield. This ratio is actually a measure of persistency rather than a numerical value for the entire shape of the lactation curve. Data were also presented showing the variation in the shape figure due to variations in service period, age, dry period, and feeding. It is concluded that the "lactation curve depends partly on a genetic characteristic of the cow."

McCandlish (11) of the Iowa Station in comparing the rate of decline of milk of the cows in the grading-up experiment has determined the persistency in terms of the percentage of the first month's production. By plotting these percentages, the difference in persistency can be noted although no numerical value is attainable. By this method it was shown that the scrub cows decline most rapidly in milk and fat secretion, indicating a decided lack of persistency, the first cross showed further improvement, and the second cross still further increase in persistency. In fact, the second cross is somewhat better in this respect than were all the pure bred cows when grouped together.

Cole (12) of the Wisconsin Station in reporting on the results of the Angus-Jersey cross stated that "the meagre records at hand indicate a milk production of the cross-breds, intermediate between that of the two original breeds, but with high production tending to be dominant. The higher production of the Jersey is due to both a greater milk flow and a more sustained lactation period. In both these characteristics the cross-breds resembled more nearly the Jersey."

QUANTITATIVE MEASURES OF PERSISTENCY

In order to study the inheritance of the character of persistency, two methods were devised which furnish quantitative measures of the declining segment of the milk secretion curve. They have already been described (13). A brief discussion of the methods follow:

At any given level of maximum milk or fat secretion, the cause of variation in total yield of milk or fat is due to variations in the rate of decline or persistency of secretion. In other words, if two cows each produce 60 pounds of fat during their maximum month and one cow produces 500 pounds of fat during the year and the other only 400 pounds of fat, it is quite obvious that the fat production of the first cow declined at a slower rate and, therefore, was more persistent.

The relation of maximum production to total production should, therefore, give an indication of the persistency of milk secretion during the lactation period. This may be illustrated as follows: If the total yearly production of a cow were 600 pounds of fat and the fat production during the maximum month were 50 pounds, then the ratio $\frac{600}{50}$ would be 12. The ratio 12 indicates that each month's production was 50 pounds, on the average, and that the cow was 100 per cent persistent. The relation

TABLE 1

Relation of per cent of persistency to the persistency ratio

PER CENT OF PERSISTENCY	RATIO OF $\frac{\text{TOTAL PRODUCTION}}{\text{MAXIMUM PRODUCTION}}$
100	12.0
99	11.4
98	10.9
97	10.5
96	10.0
95	9.6
94	9.2
93	8.8
92	8.4
91	8.1
90	7.8
89	7.5
88	7.2
87	6.9
86	6.6
85	6.4

TABLE 2

Range of persistency of fat secretion of Guernsey cows

PERSISTENCY RATIO $\frac{\text{TOTAL YEARLY FAT}}{\text{MAXIMUM MONTHLY FAT}}$ CLASS INTERVAL	NUMBER OF COWS, FREQUENCY	PERCENTAGE DISTRIBUTION	CORRESPONDING PERCENTAGE OF PERSISTENCY
5.0 to 5.4	4	0.11	
5.5 to 5.9	3	0.08	
6.0 to 6.4	8	0.21	
6.5 to 6.9	26	0.68	86.3
7.0 to 7.4	112	2.94	88.1
7.5 to 7.9	221	5.81	89.9
8.0 to 8.4	346	9.09	91.3
8.5 to 8.9	486	12.76	92.7
9.0 to 9.4	649	17.05	94.2
9.5 to 9.9	658	17.28	95.3
10.0 to 10.4	692	18.18	96.5
10.5 to 10.9	472	12.40	97.7
11.0 to 11.4	120	3.15	98.6
11.5 to 11.9	9	0.24	99.5
	3,806		

Statistical constants:

Mean persistency ratio.................................... 9.392 ± 0.0115

Standard Deviation.................................... 1.033 ± 0.00081

Co-efficient of variation.................................... 10.99 ± 0.0849 between other percentages of persistency and the ratio of total production to maximum production is given in table 1.

To gain an idea of the variation in persistency of Guernsey cows on Advanced Registry test, table 2 is presented. It is much greater than one might expect. The greatest frequency is within the range of 10.0 to 10.4 ratio of persistency. This is equivalent to about 96.5 per cent of persistency. However, over 100 animals were found to come within the 7.0 to 7.4 ratio class at one extreme and 11.0 to 11.4 ratio at the other. With cows producing 50 pounds of fat during the maximum month this means variation in yearly production of 350 to 550 pounds of fat.

THE CAUSES OF PERSISTENCY

Why do cows vary in the rate of decline of milk secretion even when producing the same yield of milk during the period of maximum production and under similar conditions of feeding and management? Even though it is a heritable characteristic, its physiological basis is of extreme interest. A number of theories may be advanced simply to point out the character of the problem being considered rather than with the idea of drawing any conclusions.

The similarity of the declining curve of milk secretion to the rate of change of certain chemical reactions has already been pointed out (8). Milk secretion even though a very complicated physico-chemical process might follow a simple chemical law if governed by the slowest process involved.

As the stimulus for the growth of the mammary gland and the secretion of milk has been quite definitely proven to be in the nature of a hormone, it appears that the decline in secretion may be due to a decline in the amount of hormone produced during the course of the lactation periods or to the gradual use of a supply stored in the body during pregnancy. Another possible cause of decline in milk secretion may be a general decline in the activity of the cells of the mammary gland or the inactivation of a progressively increasing number of the cells during the course of the lactation period. The fact that frequent milking prolongs lactation and irregular milking tends to cause a more rapid decline in milk flow might be taken to indicate that the cells become inactive because of back pressure in the gland.

Whatever the cause of persistency may be, it appears obvious that it has an important influence on lactation milk yield and is a very desirable characteristic to maintain in dairy cattle.

RELATION OF PERSISTENCY OF SECRETION TO CONFORMATION OF DAIRY COW

As persistency of milk secretion has a profound influence on total yearly production it is of interest to point out its relation to the conformation of the cow. Are there any physical indications in the conformation of the cow by which her persistency of secretion may be noted? If there are none, it would appear that conformation will never be found to indicate production closely.

The best indication of persistency of secretion in the appearance of the dairy cow may be what the judges of dairy cattle call "dairy temperament." The condition of the lean angular cow may be the result of lack of sufficient feed as well as the stimulation to continued production. This phase of the question is deserving of further attention of workers attempting to correlate type or conformation and yearly production.

COMPARISON OF GUERNSEY SIRES ON BASIS OF DAUGHTER'S AVERAGE PERSISTENCY RATIO

The records of fat production of the daughters of each Guernsey sire were studied to determine the persistency of fat secretion as indicated by the ratio of the fat production during the best (maximum) month to the total fat production. This ratio was compared with that obtained in a similar wayfrom the dams yearly fat record wherever available. No correction has been made for age or for length of pregnancy. Since completing the tabulation of this data a study was made of the influence of age on persistency. It was found that as animals grow older they decline slightly in persistency of milk secretion (6).

While pregnancy affects the persistency of secretion, it is not possible to make a correction by the method employed to deter-

TABLE 3

NAME AND NUMBER OF SIRE	DAUGHTERS									
	Number of yearly record daughters	Average mature equivalent of fat	Number of daughters compared	Maximum monthly fat production	Ratio $\frac{\text{Total}}{\text{Maximum}}$	Number of yearly record dams	Average mature equivalent of fat	Number of dams compared	Maximum monthly fat production	Ratio $\frac{\text{Total}}{\text{Maximum}}$
		pounds		*pounds*			*pounds*		*pounds*	
Brookmeads White Face, 32211	17	716	10	67.7	10.814	13	499	13	53.1	9.465
Don Bernardo of Linda Vista, 20617	11	629	9	59 3	10.660	7	527	7	56.6	9.430
Ultra May King, 27600	17	707	13	66 5	10.611	15	616	15	63.2	9.721
Langwater Cavalier, 21012	21	705	16	66 5	10.528	10	606	10	70.8	8.575
Itchen, Red Raider, 27342	10	549	7	54.3	10 517	8	634	7	62.7	9.909
Barrington May King, 19312	19	594	19	57.3	10 376	17	570	17	57.0	9.982
Allenwood King Regent, 23611	13	562	13	54.2	10 354	13	508	13	52 9	9.697
Langwater Holliston, 28055	10	726	8	70.3	10.322	6	583	6	60.9	9.542
Langwater Royal Master, 23663	14	632	10	61 5	10.254	9	626	9	65.0	9.565
Jardinieres Masher, 20957	14	668	10	64 7	10.248	11	655	11	66.6	9.844
Glenwood's Son of Gayhead, 19617	10	551	6	51.9	10.210	3	554	3	60.3	9.173
Rosies Golden King of Oakhurst, 31630	10	639	8	64.4	10 208	9	615	9	60.1	10.248
Langwater Stars & Stripes, 21822	14	708	13	70.5	10.185	10	559	10	56.2	9.875
Jewel's Independence, 10324	10	626	10	61.2	10.182	6	553	6	57.2	9.642
Lady Smith's Cherub, 30760	12	688	11	68 6	10.154	10	614	10	65.1	9.423
Governor II of the Gree, 19123	15	500	15	49.3	10.150	2	519	2	58.2	8.908
Jethro Bass, 11366	30	612	27	60.7	10.149	26	565	26	57.5	9.872
Langwater Hayes Rosies King of the May, 16723	27	681	27	67.0	10.135	22	602	22	64.1	9.382
King of the May, 9001	33	708	31	69.8	10.125	25	625	25	64.4	9 664
Rex of Rich Neck, 31472	15	645	13	64 1	10 119	13	669	13	65.2	10.241

Itchen May King, 25174	20	574	19	56 9	10 108	17	599	17	63.5	9.484
Reservation Chesterfield, 36609	13	634	9	62 5	10 106	12	556	12	58.8	9.529
Overture of Prospect, 32821	10	456	9	45 1	10 067	6	395	5	41.6	9.686
Langwater Fisherman, 21873	12	549	9	55 4	10 053	6	512	6	53 8	9.604
Hayes Cherub 2d, 25147	16	630	16	62 5	10 047	9	595	9	65 2	9.371
King Masher 8th 20973	11	595	10	59 1	10 046	9	537	9	56 5	9 519
Langwater Warrior, 26509	23	701	12	69 2	10 044	18	764	18	76 7	9.941
Vaillant Coeur, 7749	13	416	13	41 5	10 038	10	441	10	50 3	8.966
Brockton of the Glen, 15739	10	667	8	64.1	10 032	7	623	7	52 4	9.986
Pencoyds Golden May Secret, 39626	14	627	13	63.2	10 016	13	569	13	57.7	9 934
Langwater Traveler, 38325	15	678	9	65.3	10.009	11	529	10	61.0	8.676
Langwater Rival, 14194	16	639	10	63 3	9 988	10	596	10	60.4	9 874
Gold Lassie's Julian, 27704	18	674	14	68 6	9.990	18	624	18	65.4	9.791
Border Raider, 22243	30	618	26	61 6	9 980	28	566	28	69.7	9.483
Langwater Royal 7th, 20632	18	588	11	56 9	9 976	6	455	5	54 6	8.629
May Rose King, 8336	21	632	21	59.1	9.975	18	532	18	55 1	9 625
May Rose King 2d, 13130	16	594	14	61 5	9.963	9	582	9	64.2	9 052
Casterilius of Nelsonville, 18052	10	587	10	59 5	9 954	10	576	10	64.2	9.219
Beda's May King, 11893	41	640	35	63 1	9 950	31	592	30	60.3	9.748
Rhea's King of the May, 14368	21	514	20	52 4	9.941	15	511	15	57 7	8 869
The Conqueror II, 15323	17	542	17	54 7	9 929	4	504	4	53.8	9.276
Langwater Golden Secret, 26510	14	579	14	58 8	9 927	8	615	8	68.5	9.174
Julian of Koshkonong Place, 14409	10	598	10	60 6	9 905	8	548	8	58 9	9.515
Uncle Jim, 16740	17	540	15	55 7	9.896	15	574	15	61.2	9 242
Don Diavolo of Linda Vista, 23565	11	635	10	62 5	9.878	8	584	8	59.5	9 808
May King's Vrangue of Ingleside, 15430	37	634	33	63 6	9.877	30	611	30	63.2	9.584
Lavanton, 11611	10	729	10	74.6	9 873	10	690	10	73.8	9.375
Robert's Criterion of Belleview, 26887	18	589	16	58.1	9 871	2	514	2	60.7	8.395
Alderney II, 2215G	19	582	18	55 1	9 870	1	463	1	55.1	9 871
Langwater Demonstrator, 16451	66	611	51	57 9	9 865	46	515	46	55.4	9.392

TABLE 3—*Continued*

NAME AND NUMBER OF SIRE	DAUGHTERS									
	Number of yearly record daughters	Average mature equivalent of fat	Number of daughters compared	Maximum monthly fat production	Ratio $\frac{\text{Total}}{\text{Maximum}}$	Number of yearly record dams	Average mature equivalent of fat	Number of dams compared	Maximum monthly fat production	Ratio $\frac{\text{Total}}{\text{Maximum}}$
		pounds		*pounds*			*pounds*		*pounds*	
Ivy's Emblem, 3804G	10	565	9	56.5	9.864	1	662	1	70.1	9.435
Dan Patch of the Isle, 21293	11	517	11	52.6	9.850	11	511	11	65.6	9.251
Governor of the Carteret, 3421G	13	569	10	54.2	9.849	2	597	2	57.0	10.453
Jacqueminot of Linda Vista, 23564	20	537	8	55.1	9.840	12	522	12	55.2	9.544
Holden IV, 12179	12	621	10	67.6	9.838	7	689	7	74.1	9.314
May King of Linda Vista, 17946	16	599	16	61.7	9.838	15	585	15	64.6	8.828
Yeoman's King Victor, 22295	15	539	14	55.8	9.837	15	576	15	62.3	9.295
Langwater Pencoyd, 21830	18	658	15	66.8	9.837	16	679	15	69.4	9.757
Gay Boy of the Isle, 16998	17	581	17	59.6	9.836	9	571	9	64.5	8.978
Itchen Daisy's May King of Langwater, 17349	24	637	21	64.2	9.829	18	530	18	56.0	9.486
Governor I. of the Chene, 10563	24	639	24	65.2	9.811	12	671	12	68.0	9.856
Valentine's Honour of the Passee, 3784G	13	640	8	68.8	9.789	1	695	1	70.0	9.937
Bell-Founder, 11681	19	694	19	70.7	9.776	10	643	10	66.8	9.763
Ne Plus Ultra, 15265	46	687	41	70.3	9.755	32	569	32	59.7	9.551
France's Jewel's Champion, 17970	13	554	11	60.4	9.751	1	513	1	50.5	10.140
Souvenir of L'Etiennerie, 21925	17	523	17	53.8	9.747	6	584	6	63.7	9.124
Bell Buoy of Linda Vista, 19430	32	575	32	59.0	9.747	24	542	23	64.8	8.489
Dandy of Beirnetown, 29963	17	523	15	52.7	9.742	16	512	14	48.2	9.685
Frank Rilma, 21901	11	520	11	53.5	9.732	8	485	7	50.2	9.794
Lohier's Beta Raider of Waddington, 35442	20	516	15	52.8	9.731	12	496	12	63.5	8.017

Langwater May King, 13001	20	590	20	60.5	9.731	16	592	16	60.8	9.724
Pretoria's King of Midlothian, 22641	10	601	7	62.8	9.705	10	569	10	68.7	8.516
Stranford's Glenwood of Pinehurst, 13609	14	565	14	58.5	9.669	14	566	14	64.1	8.890
May Rose Secret of Pencoyd, 27844	14	549	13	56.6	9.668	9	593	9	62.0	9.656
Laverna's Ultra May King, 24660	12	585	9	63.8	9.667	7	565	7	65.6	9.463
Raymond of Blaye Farm, 191A	13	521	9	52.6	9.665	1	483	1	54.8	8.798
Langwater Islander, 31329	10	530	8	55.6	9.658	7	502	7	57.5	8.732
King of Chilmark, 20798	24	689	19	70.6	9.657	7	612	7	66.2	9.196
Bob Rilma's Monogram, 29095	10	608	5	62.8	9.653	10	523	10	59.5	8.872
Golden Secret of Lilyvale, 10028	23	674	22	70.3	9.645	16	592	16	64.6	9.201
Reputation of Portage, 10695	11	632	11	66.6	9.642	5	584	5	68.2	8.973
Dimple Bloom, 14369	10	557	10	57.4	9.637	2	584	2	60.1	9.679
Langwater Raritan, 17052	17	566	10	55.2	9.632	17	499	17	52.7	9.423
Dolly Dimple's May King of Langwater, 12997	10	630	10	65.3	9.630	9	626	9	63.1	9.682
Ledyard Bay, 11074	10	645	10	67.2	9.625	9	498	9	48.5	10.106
Prince of Sarnia, 22000	21	523	17	55.3	9.621	9	492	9	50.6	9.763
Galaxy's Lavinius, 12548	20	435	20	45.6	9.616	17	463	17	53.4	8.732
Moonlight King of Anna Dean Farm, 29586	13	543	9	57.2	9.615	5	438	5	57.8	7.325
Langwater's Hambro, 21011	27	551	26	58.5	9.605	23	482	23	53.1	9.226
Masher's Sequel, 11463	70	511	69	55.0	9.600	17	501	17	58.4	8.667
Dairymaid's Pride of Iowa, 14941	15	518	15	53.9	9.596	5	601	5	68.7	9.095
Rouge II's Son, 18587	10	471	9	49.3	9.592	9	462	9	53.6	8.756
Norfolk Squire, 11565	14	504	14	52.6	9.584	6	506	6	56.1	9.055
Rockingham, 18120	10	578	10	60.2	9.565	6	540	6	56.8	9.674
Ne Plus Ultra 4th, 29328	23	707	17	74.3	9.565	12	576	12	59.5	9.730
Pencoyd Quaker Boy, 35552	10	556	7	55.9	9.562	2	559	2	57.0	9.807
Raymond of the Preel II, 13387	12	475	12	50.3	9.651	7	538	7	58.2	9.145
Golden Secret of Pencoyd, 23462	22	536	21	56.1	9.561	22	587	21	58.1	9.924
Masher's Galore, 8572	22	615	22	64.4	9.551	15	470	15	50.9	9.320
Lord Mar, 14359	30	558	30	58.6	9.543	5	669	5	81.9	8.184

TABLE 3—*Continued*

NAME AND NUMBER OF SIRE	DAUGHTERS									
	Number of yearly record daughters	Average mature equivalent of fat	Number of daughters compared	Maximum monthly fat production	Ratio $\frac{\text{Total}}{\text{Maximum}}$	Number of yearly record dams	Average mature equivalent of fat	Number of dams compared	Maximum monthly fat production	Ratio $\frac{\text{Total}}{\text{Maximum}}$
		pounds		*pounds*			*pounds*		*pounds*	
Beau Regal, 13448	13	561	11	58 5	9.532	4	763	4	73.0	10.528
Sequel's Monogram, 15649	30	543	30	56 9	9.526	11	460	11	56.2	8.878
Florham Golden Lad, 18119	12	571	11	60.1	9 525	7	747	7	71.1	10.496
Pride of Day, 17126	14	529	14	56 0	9 515	8	540	8	59 0	9.142
Glenwood's Main Stay 40th, 28108	12	536	8	59.9	9.509	3	523	3	63.7	8.245
King Bell, 13482	10	707	10	75 0	9 501	8	581	8	59.8	9.908
Governor of the Chene, 1297G	111	524	110	55.7	9 500	2	545	2	59.2	9.123
Yeoman's King of the May, 17053	84	610	72	63.5	9.487	64	508	63	58 3	8 850
Goldseeker of Anna Dean Farm, 126106	18	547	17	57.7	9.479	10	726	10	70.6	10.047
Baubigny's Squire of Keewaydin, 21834	11	577	8	60 9	9.476	10	578	10	68.8	8.408
Rinaldo, 8917	11	601	9	63.6	9.474	11	639	11	65.2	9 844
Pencoyd's Golden Secret, 16550	14	538	12	58.3	9.474	10	612	10	63.7	9.668
Triple Champion, 13067	18	544	18	58 0	9.473	13	516	13	60.3	8.476
George Washington of Fairfield Farm, 10866	12	516	12	54 5	9 459	3	548	3	60.8	9.051
Nelson II, 52A	12	497	12	53 4	9.452	1	476	1	54.8	9.861
Duke of Mouilpied, 2045G	11	523	11	55 2	9.450	1	408	1	48.8	8.340
Raymond of the Preel IV, 19235	17	505	17	54.4	9.445	3	399	2	44.3	8 984
Capt. Robbie, 7146	11	442	11	47.4	9.435	10	484	10	53.4	9.212
St. Austell Dreadnaught, 34671	10	501	10	53.8	9.429	9	502	9	54.4	8.814
Fanny's Sequel, 19563	28	478	27	49 9	9.428	7	574	7	61.1	9.459

Charmante's Rose King, 11746	11	624	11	66.0	9.422	7	532	7	57.3	9.334
Jethro's May King of Linda Vista, 14591	15	620	12	63.6	9.420	15	615	15	62.9	9.843
Cherub's Winner, 34180	14	575	12	60.6	9.417	11	491	11	59.0	8.324
Duke of Waumesit, 13480	12	555	12	59.0	9.414	6	494	6	53.9	9.197
Malcolm of Maplehurst, 5626	12	454	12	48.7	9.412	7	512	7	50.9	10.035
Penwyn of Rosendale, 11282	11	566	11	60.4	9.404	8	683	8	69.5	9.945
Flora's Sequel of Vimiera, 25905	20	572	20	60.8	9.397	1	526	1	60.7	8.662
Sailor Lad of the Fontaines, 51090	17	605	14	63.1	9.395	2	599	2	62.1	9.803
Christie's Combination, 14651	10	475	10	51.2	9.389	9	427	9	46.2	9.370
Demonstrator of Roughwood, 23133	15	624	11	65.6	9.388	7	561	7	66.3	8.567
Jewel's Royal Combination of Wawa, 15655	11	588	10	63.2	9.374	9	493	9	53.1	9.413
General Bay, 16177	11	506	9	56.0	9.372	10	621	10	65.0	9.708
Marcia's Glenwood of Pinehurst, 11560	10	545	9	58.7	9.365	6	514	6	54.8	9.480
Victor of Pencoyd, 18901	18	575	16	62.9	9.360	16	546	16	54.1	10.123
Spotswood's Rival, 8346	12	581	10	59.7	9.354	2	471	2	44.1	10.643
Galaxy's Sequel, 16904	53	523	51	56.4	9.354	13	542	13	60.5	9.001
Raymond's Pioneer of Lewiston, 19103	20	590	19	56.3	9.351	14	617	14	66.5	9.243
Glenwood's Stranford, 9386	20	516	20	55.0	9.349	14	448	14	48.7	9.270
Roy of Norwood, 8141	18	530	18	56.7	9.344	8	470	8	51.7	9.057
Princess' Jewel, 24877	31	515	31	55.3	9.343	3	550	3	57.1	9.652
Frank Rose, 26342	11	562	10	60.3	9.332	9	483	9	51.9	9.328
Hope's May Rose of Maple Hill, 35903	12	518	12	55.6	9.329	7	447	7	57.8	7.795
Florham Laddie, 20431	30	662	26	69.6	9.314	11	592	11	62.5	9.492
Golden Anne's Fernwood of Homestead, 11916	16	461	15	49.1	9.314	2	518	2	50.0	10.268
Glenwood's Main Stay 16th, 9384	10	597	10	64.9	9.313	6	486	6	51.1	9.154
Sunburst's King Masher, 37423	16	420	15	44.9	9.305	12	421	11	46.3	8.950
Strong Anchor, 5849	15	491	15	53.2	9.296	3	441	3	45.8	9.608
Begalore, 10101	13	540	13	57.8	9.291	6	570	6	59.8	9.461
Glenwood's Main Stay, 6067	26	482	26	53.0	9.290	16	440	16	49.0	9.077
Governor of the Vanquiedor, 39925	20	508	18	56.6	9.284	2	483	2	60.1	8.036

TABLE 3—*Continued*

NAME AND NUMBER OF SIRE	DAUGHTERS									
	Number of yearly record daughters	Average mature equivalent of fat	Number of daughters compared	Maximum monthly fat production	Ratio $\frac{\text{Total}}{\text{Maximum}}$	Number of yearly record dams	Average mature equivalent of fat	Number of dams compared	Maximum monthly fat production	Ratio $\frac{\text{Total}}{\text{Maximum}}$
		pounds		*pounds*			*pounds*		*pounds*	
Endymion, 8916	16	533	16	57.0	9.283	8	568	8	59.3	9.550
Robert's Boy, 21662	13	520	12	55.0	9.277	3	568	3	64.0	8.710
Northern Boy, 1779G	11	448	11	52.7	9.268	1	621	1	72.5	8.558
Alderney Raymond, 26357	29	516	28	56.0	9.244	2	640	2	64.8	9.891
Buckthorn, 4781	11	547	11	58.8	9.241	3	525	3	53.6	9.852
Golden Nobel II, 1836G	29	590	29	64 3	9.241	3	447	3	56.1	7.980
Sister's King, 33653	11	562	9	60.7	9.236	4	423	3	52.9	8.195
Golden Secret, 12599	15	529	15	56.5	9 218	7	507	7	57.5	8.978
Pretor, 9316	14	524	14	57.6	9.211	4	519	4	56.1	9.198
Marshall of France, 9051	12	549	12	60.4	9.206	5	506	5	53.4	9.560
Glenwood's Combination 8th, 12550	11	492	11	54.8	9.205	5	438	5	46.4	9.510
Dairymaid's King, 12898	21	490	21	53.4	9 200	18	438	18	54.8	8.113
Langwater Peerless, 19227	12	559	10	62.5	9.181	7	613	7	63.5	9.657
Christy of Pinehurst 31st, 31984	10	479	8	51.6	9.177	5	419	5	48.8	8.577
Ukiah, 14344	14	405	14	45 1	9 174	14	447	14	48.5	9.282
Florham Monarch, 20771	10	575	9	61.5	9.172	9	599	9	66 9	8.894
Langwater Frenchman, 19226	28	537	25	57.7	9.171	24	542	23	58.6	9.261
Dairymaid's Criterion of Iowa, 28187	12	598	11	65.9	9.160	6	523	6	57.9	9.228
Spotswood Sequel, 9686	30	532	28	62.1	9.160	17	502	17	55.5	9.133
Itchen May King of Stannox, 34377	13	534	11	58 2	9.158	12	591	11	64.8	9.028

King of Medfield, 15434	10	549	10	59.9	9.153	4	431	4	52.0	8.200
Glenwood's Reputation, 7687	20	533	20	58.9	9.152	4	477	4	52.2	9.196
Glenwood Boy of Haddon, 4605	26	521	26	57.3	9.147	21	559	21	64.8	8.613
Lily Ella's Squire, 6597	15	494	15	54.5	9.144	5	456	5	46.1	10.219
Dean of the May, 21815	20	526	17	55.7	9.135	14	547	14	57.8	9.556
Cora's King of Belleview, 9779	11	569	11	62.5	9.114	3	494	3	63.5	7.804
Skeezicks, 9979	13	483	13	53.7	9.113	9	537	9	65.3	8.148
Langwater Royal, 14253	27	616	25	66.8	9.107	22	554	22	62.2	8.889
Sir Snowdown, 19252	20	496	19	53.5	9.104	17	431	17	47.6	9.094
Auricula's Main Sheet, 8870	10	495	10	55.0	9.099	5	528	5	53.5	9.821
Honoria's Sequel II, 40668	16	568	13	63.1	9.093	2	580	2	55.9	10.394
Aimable of France, 13739	10	552	10	60.7	9.076	5	496	5	54.6	8.762
Langwater Frederick, 22268	17	553	15	59.0	9.076	12	592	12	61.4	9.694
Langwater Dictator, 15668	11	513	11	56.9	9.062	9	421	9	49.6	8.387
Gay Lad de Braye, 2026G	18	543	18	60.1	9.059	1	648	1	60.7	10.669
Lord Waukesha, 1014G	18	575	18	64.3	9.056	14	522	14	59.1	8.881
Jessy Rose's Pride of Iowa, 23955	19	519	17	63.9	9.056	14	504	14	53.9	9.319
Modena's Yeoman of Langwater, 10764	15	553	15	61.4	9.050	2	642	2	71.9	8.941
Royal Governor of L'Etiennerie, 1484G	10	547	10	60.1	9.023	1	487	1	67.3	7.246
King's Vanguard, 22719	13	627	13	69.7	9.021	11	524	11	62.4	8.486
Spotswood Masher Sequel, 9687	11	504	11	55.7	9.017	7	444	7	54.0	8.355
Onoko of Maple Row, 11522	10	537	10	61.8	9.014	8	533	8	58.8	9.162
Lehigh's Golden Emperer, 15761	18	516	15	57.2	8.999	6	458	6	60.4	7.616
Rutila's Sheet Anchor, 5701	11	505	11	56.2	8.984	4	472	4	51.8	9.046
Coralette's Son, 3987	12	399	12	44.9	8.976	11	421	11	51.9	8.167
Preel VIIs Champion Butter Boy, 26132	13	425	12	46.2	8.960	12	448	12	47.2	9.573
Starlight's Excelsior, 7992	24	521	24	58.3	8.956	10	477	10	57.9	8.265
France's Masher 2d, 7248	12	512	12	57.3	8.954	1	649	1	68.2	9.511
Barmouth Pencoyd, 11059	12	461	12	52.0	8.954	6	564	6	64.0	7.971
Raymond of the Preel, 11353	17	451	17	51.5	8.950	4	476	4	54.7	8.733
Beda's May Day, 34995	10	546	9	55.3	8.946	7	593	7	68.2	8.702
Selma's Glenwood, 12596	19	478	19	53.8	8.937	15	526	15	60.7	8.809

TABLE 3—*Concluded*

NAME AND NUMBER OF SIRE	DAUGHTERS									
	Number of yearly record daughters	Average mature equivalent of fat	Number of daughters compared	Maximum monthly fat production	Ratio $\frac{\text{Total}}{\text{Maximum}}$	Number of yearly record dams	Average mature equivalent of fat	Number of dams compared	Maximum monthly fat production	Ratio $\frac{\text{Total}}{\text{Maximum}}$
		pounds		*pounds*			*pounds*		*pounds*	
Giltedge of Koshkonong Place, 21989	21	538	19	61.8	8.913	20	487	20	52.7	9.169
Lord Mar V, 18961	10	456	9	53.4	8.890	1	408	1	42.5	9.589
Moss Raider, 22155	15	536	14	60.5	8.853	9	483	9	54.0	8.980
Jury of Koshkonong Place, 16793	24	497	23	56.8	8 833	16	451	16	49.4	9.180
May King of Ingleside, 12558	22	521	22	59 7	8 829	20	483	20	55.0	8.888
Dairymaid's Standard of Iowa, 28946	12	541	11	58.0	8.816	11	533	11	58.2	9.272
Joker of Riverside, 21447	10	595	10	67 5	8.796	4	558	4	62.8	8.887
King Masher, 11048	23	665	15	75.0	8.739	16	593	15	62.9	9.585
Masher of Sarnia, 19167	11	541	9	62.6	8.730	3	564	3	60.3	9.376
Golden Hero of L'Etiennerie, 12647	16	553	15	64.0	8.698	5	461	5	59.5	7.899
Wolfram, 5640	10	470	10	54.7	8.670	5	667	5	67.1	9.948
Raymond of the Preel VI, 14360	12	467	12	54.1	8.640	3	494	3	52.4	9.440
Guiding Star, 12423	15	486	14	56 9	8.632	11	579	11	65.1	8.914
Silver King of the Isle, 14363	13	531	13	62.2	8.613	9	517	9	55.2	9.412
Gypson's Count, 8125	18	434	18	51 0	8.569	1	416	1	51.7	8.039
Robina's Standard, 7254	21	473	21	56.1	8.515	11	535	11	59.7	9.065
Selma's Main Stay's Son, 23585	14	476	13	54.5	8.500	9	520	9	64.7	8.072
Rutila's Gold Basis, 5626	13	487	13	57.9	9.464	7	455	7	52.3	8.764
Radium, 9193	12	445	12	53.5	8.425	9	495	9	63 8	7.930
Penwyn 2d, 7559	10	400	10	49.5	8.213	5	428	5	58.1	7.462
Ideal's Senator, 14736	13	474	13	58.5	8.185	11	454	11	54.7	8.374
Mars of Woodcrest, 9290	10	563	10	69.7	8.070	4	459	4	57.6	7.934

mine persistency. The data may, therefore, be criticized from this standpoint. It is not believed that the total effect of these factors, however, would greatly alter the results (3).

An examination of table 3 will reveal the fact that large maximum production of fat is not necessarily associated with persistent production. This indicates the necessity, not only of determining that a sire is producing daughters possessing the ability to produce large quantities of milk and fat during a short time but that it is equally important that the daughters possess the ability to maintain that production at a high level for a long period.

The maximum and the persistency secretion of the dams of the daughters is also presented for each sire. Two comparisons are possible in this connection; first, the average maximum production of dams and daughters; second, the average persistency of secretion of the dams and daughters. The first comparison has previously been discussed (9). The second will be analyzed here.

COMPARISON OF THE PERSISTENCY OF SECRETION OF THE DAMS AND DAUGHTERS

In order to determine the relation between the persistency of secretion of the dams and daughters, the following method was used. The dams and daughters persistency ratios were compared within groups of sires having similar daughter averages. This method was employed in order to make fairly constant the influence of the sires. Within each group of sires the average ratio of the daughters by dams with persistency ratios gradually increasing was determined. The increase in the ratio of the daughters for each unit increase in ratio of the dams was taken to indicate the supplementing effect of the dam on the daughter. The results are shown in tables 4 and 5. It is apparent from an examination of the tables that there is little increase in the daughters persistency with an increase in the dams persistency.

Straight line equations were fitted to the observed values of the form $D = a + bd$ in which D is the average persistency of the daughters, d the persistency of the dam, and b the constant change in persistency of the daughters for each unit of increase in the persistency of the dams above *a*, the average potential transmitting ability of the sires in the group.

The values obtained for *b* in each sire class indicate the supplementing effect of the dam on the daughters persistency. The limited numbers of comparisons causes considerable fluctuation but it is obvious that the positive effect of the dams persistency on the daughters persistency of secretion is, on the average very low.

It was pointed out in the previous paper in this series that assuming dominance as the dams increase in homozygosity for the factors concerned, the greater will be the relation between her producing ability and her transmitting ability. As there has been little selection on the part of breeders for increased persistency of their animals might not the results obtained be explained by the extreme heterzygosity of the dams for persistency. It is apparent that the persistency of the dam as measured by her record of production does not supplement the sires potential transmitting ability for persistency. In other words the phenotype of the dam is not a satisfactory measure of her genotype.

TABLE 4

CLASS OF SIRES	CONSTANTS
Sire's daughter's persistency ratio	
above: 10.5	a = 11 59, b = −0 100
10.00–10.49	a = 9 35, b = +0.081
9.75– 9 99	a = 9.63, b = +0.030
9.50– 9.74	a = 10 20, b = −0 065
9.25– 9.49	a = 8.56, b = +0 099
9 00– 9.24	a = 8 66, b = +0 055
8 50– 8 99	a = 9.23, b = −0.033
8.00– 8 49	a = 8.72, b = −0.046

As a genetic interpretation of the results of this and previous studies of the mode of inheritance of milk and fat secretion is being made in connection with a study of the progeny performance of Jersey sires and dams which is being submitted for publication to the Missouri Agricultural Experiment Station, the theoretical aspects of the problem will not be discussed in the present paper.

TABLE 5

Dam and daughter comparison of Guernsey sires

DAMS PERSISTENCY RATIO	SIRES WITH DAUGHTERS PERSISTENCY RATIO BETWEEN 8.0–8.49		SIRES WITH DAUGHTERS PERSISTENCY RATIO BETWEEN 8.5–8.99		SIRES WITH DAUGHTERS PERSISTENCY RATIO BETWEEN 9.00–9.24		SIRES WITH DAUGHTERS PERSISTENCY RATIO BETWEEN 9.25–9 49		SIRES WITH DAUGHTERS PERSISTENCY RATIO BETWEEN 9.50–9.74		SIRES WITH DAUGHTERS PERSISTENCY RATIO BETWEEN 9 75–9 99		SIRES WITH DAUGHTERS PERSISTENCY RATIO BETWEEN 10.0–10 49		SIRES WITH DAUGHTERS PERSISTENCY RATIO ABOVE 10.5	
Class	Pairs	Daughter average ratio	Pairs	Daughter average ratio	Pairs	Daughter average ratio	Pairs	Daughter average ratio	Pairs	Daughter average ratio	Pairs	Daughter average ratio	Pairs	Daughter average ratio	Pairs	Daughter average ratio
6.5– 6.9															1	9 81
7.0– 7.4	5	9.03	12	9.13	15	9 29	15	8.85	9	9.99	5	9.60	3	9 09	1	11 14
7.5– 7.9	10	7.98	15	9 02	32	9.03	22	9.26	23	9 69	22	9 93	6	10 30	2	10 33
8.0– 8.4	7	8 90	32	8 78	43	8.92	46	9 73	41	9 32	35	9 97	11	10 00	4	10 66
8.5– 8.9	2	7.52	31	9.08	55	9.15	55	9 53	51	9 71	39	9.81	28	10 02	8	10.62
9.0– 9.4	7	8.78	42	8 91	62	9.18	65	9 53	60	9 58	69	9 83	47	10 19	5	10 88
9.5– 9.9	6	8 29	30	9 08	48	9.17	59	9 39	55	9 55	66	10 07	52	10 10	8	10.43
10.0–10.4	5	7.76	30	8 53	34	9.20	59	9 49	48	9.68	72	10 01	68	10 08	4	10.55
10.5–10.9			5	8.96	13	9.28	41	9.53	38	9 38	42	9.75	29	10 18	4	10.72
11.0–11.4			3	9 23	4	8 85	10	10 00	5	9.90	9	9 96	13	10.40		

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"VISCOLIZED" MILK AND ITS DETECTION*

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The practice of separating a portion or all of the cream from milk, homogenizing (viscolizing) it, and later remixing it with the remaining milk and skimmilk or with skimmilk only as the case may be, has been receiving considerable attention. This practice causes an increase in the volume of cream rising on the bottled milk, the amount of increase depending on several factors under the control of the processor.

Recently the process has been declared illegal in Pennsylvania and in a test case the court decision was against the vendor of the "viscolized pasteurized milk" who was found guilty of "intent to deceive."

In order to obtain conclusive evidence of the use of the process by a dealer the authorities charged with enforcing the food laws have found it necessary actually to see the process in use. This is not only inconvenient but may be extremely difficult where the user does not wish the fact known. It was on this account primarily that the present study was made, the object being to develop a means of detecting "viscolized" milk by some simple examination of the suspected sample.

LITERATURE

While the process used in making the so-called viscolized milk with its extended cream layer has only recently been patented, and more recently used to any note-worthy extent, it has been known, at least in a few plants, for sometime. In fact, the writer's attention was called to it by a milk dealer in 1919.

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† Assistance with the experimental work was given by J. H. Erb and F. E. Geyer.

Since that time several investigators working with other products have pointed out certain aspects of the homogenizing or viscolizing process which shed some light on the causes of the cream layer extension in "viscolized" milk.

Mortensen (1) states that homogenization of cream causes a decrease in the size of the fat globules and causes them to be brought together in large clusters, although curiously enough he attributes the increased viscosity to the increased surface of the fat rather than to the clusters themselves.

Everson and Ferris (2), Dahle and Martin (3), Sherwood and Smallfield (4), and Reid and Mosely (5) have all called attention to the fact that homogenization of cream and ice cream mixes causes, not only a subdivision of the fat globules, but also their aggregation into clumps or bunches. Most of the workers attribute the increased viscosity caused by the process to the clumps rather than to a greater surface of the fat although this is undoubtedly a minor factor.

Martin and Combs (6) were perhaps the first to definitely give data showing the effect on the volume of the cream layer of milk when such milk was made by mixing homogenized cream and skimmilk. This is essentially the process for "viscolized" milk, although in most cases only a portion of the milk is separated to produce cream for homogenization.

In order to verify these statements and to show whether they apply in the case of "viscolized" milk as well as to determine some of the factors affecting this homogenization and creaming phenomena, the following experimental work was undertaken.

EXPERIMENTAL

Characteristics of the creaming of "viscolized" milk

Fresh, raw, 20 per cent cream was homogenized at 110°F. under a pressure of 2000 pounds. This cream was standardized to 4 per cent fat with fresh, raw skimmilk and examined under the microscope to determine the condition of the fat globules. Likewise a sample of the same cream prior to viscolization was standardized to 4 per cent fat with a portion of the same skimmilk and examined, as was also a sample of fresh raw whole milk of approximately the same fat content. The results of the observations showed the fat in the first mentioned lot of milk to be very finely divided, but gathered together into clumps containing hundreds of small globules. These clumps were extremely numerous, quite large in size and of irregular shape resembling flecks of foam. No normal globules were observable. In the other two samples the fat globules were practically identical in size and appearance, being large, mostly spherical, and individual in most cases, although a few clumps were to be seen. These clumps, however, in no way resembled the clumps in the sample reconstituted from homogenized cream, being composed of large globules whose outlines could be clearly seen. They appeared more like compact bunches of grapes and the number of globules composing them could almost be counted accurately. Usually not over a dozen or two individuals composed a clump.

The three samples of milk were allowed to cream in 250 cc. graduated cylinders for twenty-four hours at 50°F. The percentages by volume of the risen cream were as follows:

	Per cent cream
1. Homogenized 20 per cent cream standardized with skimmilk to 4 per cent butterfat	71.2
2. Unhomogenized 20 per cent cream standardized with skimmilk to 4 per cent butterfat	12.0
3. Normal unprocessed whole milk testing 4 per cent butterfat	12.8

These data show strikingly the enormous increase in the volume occupied by the risen fat in the case of the sample made from the homogenized cream. This has been interpreted as being due to the looseness of packing of the fat, when in the form of "visco-clumps," as compared with the packing of normal globules under the same conditions. That the concentration of the fat in the cream layers is much less in "viscolized" milk than in normal milk hardly needs proof. However, in the case of the three samples mentioned this fact was indirectly ascertained. Samples were allowed to cream in graduated separatory funnels under the same conditions as obtained for the cylinders mentioned previously. The volume of cream rising compared closely with the figures given above. The under layer of skimmilk was drawn off drop by drop very carefully and analyzed for fat with the following results:

	Per cent fat
Sample 1	1.08
Sample 2	1.28
Sample 3	0.99

Comparatively, there is little difference in the fat test of the skimmilk from the different samples. However, sample 1 had a volume of risen cream roughly six times as great as that of sample 2. The cream in sample 1 must then have been about one-sixth as rich in fat.

It is interesting to note that if there is any significant difference in the exhaustiveness of creaming between samples 1 and 2, it favors the "viscolized" milk. This may be due to the action of the "visco-clumps" which might be expected to carry up with them many small individual globules incapable of rising themselves. This fact may be a minor factor in the extension of the cream layer as well as the looseness of packing.

Effect of pasteurization

The three lots of milk under discussion were pasteurized in order to determine the effect of pasteurization on the cream volume of the "viscolized" milk. The process was carried out by immersing the containers in water at 160°F. until a temperature of 145°F. was attained after which they were held at this point for thirty minutes and subsequently cooled to 50°F. and held in cylinders for twenty-four hours prior to measuring the cream layers. The results were as follows:

	Per cent cream
Sample 1	40.0
Sample 2	10.8
Sample 3	10.5

Sample 1 was also examined under the microscope and the appearance of the fat compared with that in the unpasteurized portion. It was found that the "visco-clumps" were not so large and there was apparently a greater proportion of individual small globules. These facts would explain the marked decrease in the volume of the cream layer. The amount of cream rising however is still enormous compared with that of normal milk of the same fat content. Pasteurization therefore can be practiced without destroying entirely the ability of "viscolized" milk to produce an enhanced cream layer.

TABLE 1

	CREAM LAYER
Series I. Skimmilk plus	
	per cent
43.0 per cent homogenized cream	27.5
31.5 per cent homogenized cream	32.8
27.0 per cent homogenized cream	35.2
43.0 per cent unhomogenized cream	17.6
31.5 per cent unhomogenized cream	18.0
27.0 per cent unhomogenized cream	18.6
Series II. 1.7 per cent whole milk plus	
35.0 per cent homogenized cream	15.2
17.5 per cent homogenized cream	36.5
35.0 per cent unhomogenized cream	9.0
Series III. 2.2 per cent whole milk plus	
35.0 per cent homogenized cream	17.8
17.5 per cent homogenized cream	31.6
35.0 per cent unhomogenized cream	9.8
Series IV. 2.0 per cent whole milk plus	
35.0 per cent homogenized cream	20.1
17.5 per cent homogenized cream	37.7
35.0 per cent unhomogenized cream	13.2

The control of the cream layer volume

Two very simple ways of controlling the volume of cream rising on "viscolized" milk are used by the milk dealer making the product. One method, and the one usually used, is to separate and homogenize enough 20 per cent cream from the lot of milk to give the desired result. The greater the amount of cream taken off and processed and subsequently returned, the larger will be the volume of cream rising on the bottled milk. Another method is to remove a given amount of cream but vary the richness thereof. Under this condition the richer the cream viscolized the greater the final cream layer volume because a greater proportion of the fat will be subjected to the process. However peculiarly enough, the cream layer volume does not correlate directly with the amount of fat viscolized. This is shown by the data in table 1 which was obtained by reconstituting 4 per cent milk from milk and cream of five different fat contents, both homogenized and unhomogenized.

It is evident from a survey of these data that the factor exercising the greatest influence on the volume of cream rising on milk reconstituted to a uniform fat content from homogenized creams of various richness is not the amount of fat that was actually exposed to homogenization, since this was the same in each case, but curiously enough the amount of cream in which a given amount of fat was contained. This would suggest that homogenization sets up a structure of some sort among the "visco-clumps" of fat in the cream and that the volume of this structure depends on the available space at the time of homogenization and further that this structure maintains, to a certain extent, its volume upon dilution of the cream with fluid milk or skimmilk.

The data presented in this paper represent but a small portion of that obtained. However the agreement was so close in all cases with that submitted that for the sake of brevity and simplicity the rest has been omitted.

The detection of "viscolized" milk

Milk exhibiting a deeper cream layer than normal, considering the fat content, is to be suspected of being "viscolized" milk. It can readily be ascertained whether or not this is the case by a very simple microscopic examination.

The suspected sample is carefully mixed; and, using the head of a pin or similar implement, a very small portion is transferred to a drop of water previously placed on a cover glass. The sample and the water are not mixed. The cover glass is then inverted over a depression slide and viewed under the microscope. Instead of mixing the milk a sample of the cream may be taken, but in such cases the tendency is to get too much fat in a microscopic field so that globules and clumps are too close together to readily distinguish the characteristics of the sample. In cases where the mounted sample is to be held for some time, vaseline may be used between the cover glass and slide to prevent evaporation. A sample of normal milk may be viewed in the same manner for comparison although this is unnecessary after a little experience is had in knowing what to look for.

FIG. 1. THE FAT OF NORMAL MILK UNDER LOW POWER MAGNIFICATION (10 × EYE PIECE 16 MM. OBJECTIVE)

Globules large and fairly uniform in size

It is a good plan to examine the sample under the low power magnification first (10 × eye piece and 16 mm. objective). Normal milk contains fat globules which are round, mostly individual, and fairly uniform in size. Dark patches of irregular shape and size are to be taken as indications of "visco-clumps" which should, however, always be verified under a higher magnification (4 mm. objective or even the oil immersion in some cases) for confirmation. Naturally, "viscolized" milk may also contain normal globules depending on whether only a portion or all of the fat was homogenized in the form of cream. Figure 1 shows the appearance of normal fat globules under low power

Fig. 2. The Fat of "Viscolized" Milk under Low Power Magnification (10 × Eye Piece—16 mm. Objective)

This sample was reconstituted to 4 per cent from 2 per cent partially skimmed, whole milk and homogenized 35 per cent cream.

Fat clumps are plainly evident although the size of globules composing them cannot be well judged. Some normal globules from the 2 per cent milk are also present.

magnification. Figure 2 shows the appearance of the ‘visco-clumps” of viscolized milk under the same power.

In confirming the identification of “viscolized” milk under high power magnification special attention is directed to: (1) The presence of “visco-clumps” of fat, the appearance of which have already been described. (2) The appearance of comparatively large numbers of very small fat globules. Under the high power the size of the globules composing the clumps can be determined and therefore whether they are normal clumps or “visco-clumps.” Even a very few typical “visco-clumps” indicate beyond a doubt that the milk has been treated by homogenization in such a way as to extend the cream layer.

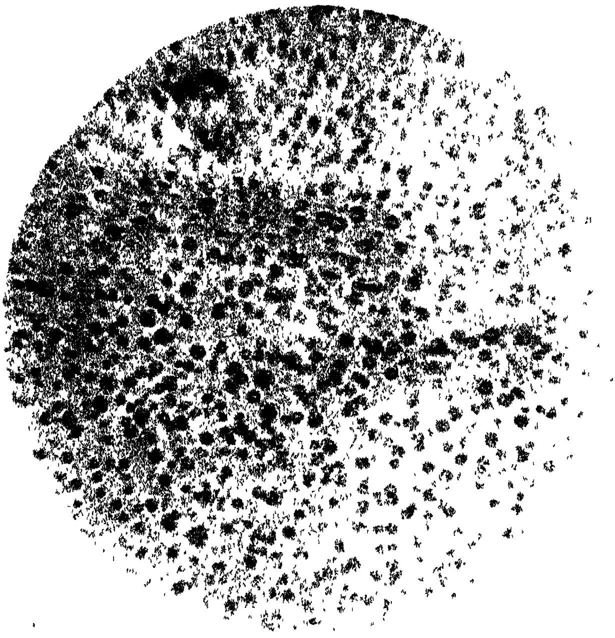

Fig. 3. The Fat of Normal Milk under High Power Magnification (10 × Eye Piece -4 mm. Objective)

Homogenization of whole milk as such not only destroys the cream layer entirely but under operating conditions, at usual pressure, does not give rise to "visco-clumps" of fat until the concentration of fat in the milk is in excess of about 8 per cent. Figure 3 illustrates normal milk under high power magnification while figure 4 shows the appearance of "viscolized" milk under the same conditions.

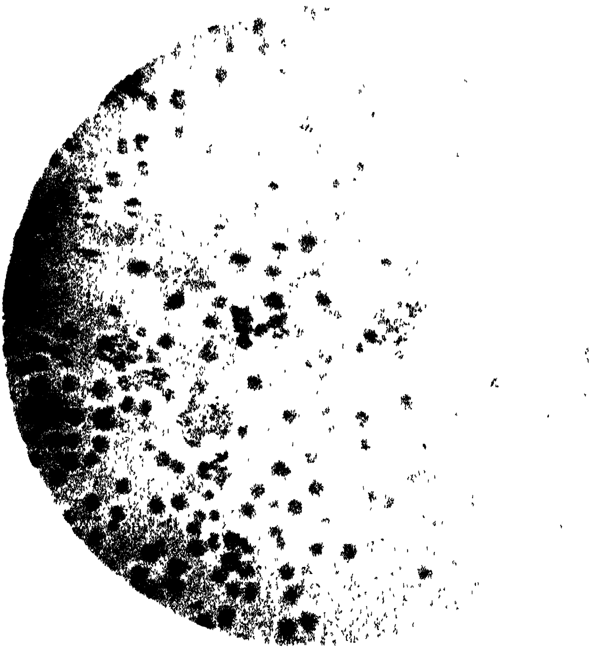

FIG. 4. THE FAT OF VISCOLIZED MILK MAGNIFIED AS IN FIGURE 3 (10 × EYE PIECE—4 MM. OBJECTIVE)

This sample is the same as no. 2. The clumps can readily be identified as true "visco-clumps" since the size of the globules composing them is infinitely smaller than the normal globules also present.

In using this method the writer was able to distinguish in all cases "viscolized" milk containing as little as 0.5 per cent (by volume) of homogenized 35 per cent cream and homogenized 17.5 per cent cream respectively. This small addition had practically no effect on the volume of risen cream so that it is confidently believed that this method of detection will apply in all cases.

SUMMARY

When fluid milk or cream containing sufficient fat (8 per cent or more) is homogenized at a pressure of 2000 to 3000 pounds per square inch a peculiar structure in the fat is created. The globules are greatly reduced in size and the myriad of small globules formed tend to clump together while the clumps themselves show a tendency to arrange themselves in such a fashion as to occupy the greatest possible space. Such milk or cream, will not separate a cream layer. If it be mixed with normal raw whole milk or skimmilk, however, a deep cream layer is formed on standing. The depth of the layer depends more on the volume of the homogenized milk or cream which was mixed with the normal skim or whole milk than on the actual amount of fat that was homogenized.

These phenomena make possible the preparation of "viscolized" milk, a product which has been and possibly is being sold in some localities, either openly or without admission. The process produces a greater cream volume on the held bottled milk than normal milk of the same fat content, and therefore deceives the purchaser who is apt to judge the richness of the product by the depth of the "cream line."

Pasteurization of such processed milk does not destroy the clumped structure of the fat although the clumps are seemingly smaller and more individual globules are present. The cream layer while considerably diminished in volume by pasteurization is still in most cases greater by far than similar normal milk.

A simple, accurate, means of detecting "viscolized" milk is described, the only apparatus needed being a microscope. A limited amount of experience, however, in judging the appearance of normal and "viscolized" milk is necessary for highly dependable results.

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A MECHANICAL DEVICE FOR INCREASING THE ACCURACY IN THE FEEDING OF HAY TO EXPERIMENTAL ANIMALS*

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The device herein described was developed to facilitate the feeding of hay without waste to experimental cows. It arose in connection with the silage feeding investigation that was begun at Storrs in 1920.

In the first five trials the experiments were so conducted that the hay consumption was restricted and uniform in amount, but as the sixth and seventh trials involved the feeding of hay for ad libitum consumption some measure was necessary to accomplish this which would at the same time curtail waste and assure accuracy. The mangers could not be relied upon to prevent an animal from appropriating a portion of its neighbors allowance when the hay is fed liberally.

Under conditions which provide a completely enclosed manger separated from the neighboring mangers, or where there is ample space between individuals such a problem does not arise. But very often it is found necessary to adapt an experiment to ordinary conditions such as were faced here. Probably the device herein described will offer suggestions for the solution of similar situations in other places, which is our reason for presenting its description.

The stanchions and mangers in use here are of the usual modern arrangement for dairy herds. The mangers are a continuous trough in front of the stalls, except for a movable metal partition. This partition prevents access by the cows to grain and silage but not of bulky hay when liberal quantities are being fed.

The experimental hay bag made to serve the purpose mentioned was constructed from 12-ounce canvas and was designed to per-

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* Being an extract from a thesis problem conducted by Mr. Connelly. Received for publication August 1, 1927.

mit the feeding of large quantities of roughage without appreciable loss; to be light and easily manipulated; and to be durable enough to withstand the usage commonly associated with dairy cattle feeding. It has met these conditions satisfactorily.

When assembled (see fig. 1) the bag is suspended from two 1½-inch horizontal pipes by 14 gauge wire hooks. The horizontal pipes are 2 feet 8 inches long and connected rigidly to the perpendicular stall posts at points *A* and *D* by 1½-inch clamp pipe sockets. Each horizontal pipe has $\frac{3}{16}$-inch drilled holes at regular intervals along its upper surface to allow easy insertion of the supporting hooks. For further security against detaching the bag by an animal, 3½-inch spring chain snaps instead of the wire hooks may be used to support the bag at points *A*, *B*, *C* and *D*. A ⅜-inch or $\frac{7}{16}$-inch hole may be drilled in the horizontal pipes at point *A* and point *B*, and closed 3-inch S-hooks may be firmly wired to the stall posts at points *A* and *D* to allow for easy attachment of the chain snaps. Chain snaps are also attached to the breast piece (*E*-*F*-*L*-*O*) of the bag at points *E* and *F*. Closed S-hooks are provided at the corresponding points on the stall posts at the stall attachments. The greatest strain is likely to be on the breast piece at points *E* and *F*, hence the necessity for snaps at

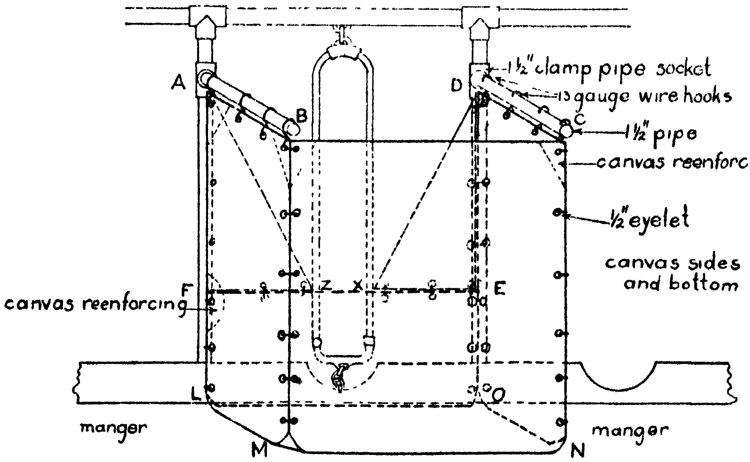

FIG. 1. FRONT ELEVATION OF EXPERIMENTAL HAY BAG

these points. Provision is made for other stall post attachments between *A-F* and *D-E*, when with certain animals such additional security is found to be necessary.

Figure 1 shows the front elevation of the bag in place before a stanchion. Parts *A-L-M-B*, *B′-M-N-C′*, *D-O-N-C*, and *E-F-L-O* (see also fig. 3) are so matched that when they are assembled *B* coincides with *B′*, *C* with *C′*, *F* with *F′*, *E* with *E′* and so on. The triangular pieces *A′-F-Z* and *D′-E-X*, as well as all the other elements of the bag, are bound into place by heavy twine bindings drawn through the coinciding $\frac{1}{2}$-inch brass eyelets. The use of twine for binding the bag parts is desirable because of the facility which twine lends to the assembly and release of the parts.

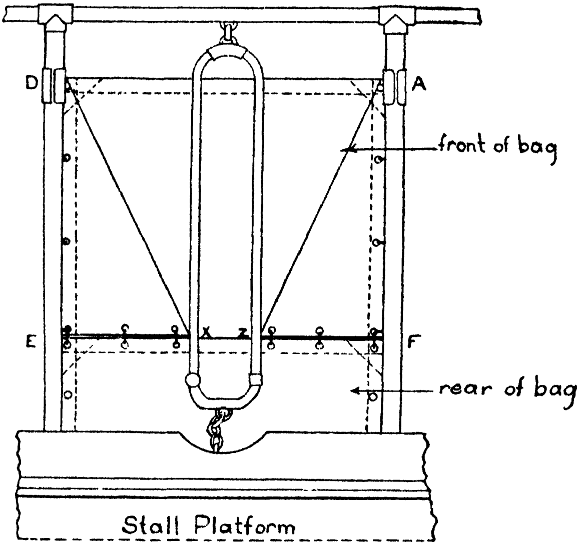

Fig. 2. Rear Elevation of Experimental Hay Bag

Figure 2 is a rear elevation of the bag in place before a stanchion. The line *E-F* is the upper edge of the breast piece. *D-E-X* and *A-F-Z* are detachable pieces and in all cases may not be necessary. These triangular pieces together with the breast piece are for the purpose of preventing the discharging of hay under the feet.

A diagrammatic sketch of the bag, designed for a standard 3-foot-8-inch stanchion, is shown in figure 3. The bag proper may be constructed from three pieces of 12-ounce canvas. The two sides and bottom (*A-B-C-D*) form one piece (12 feet 2 inches by 2 feet 6 inches). In addition an allowance of 2½ inches for lapping along the reenforced edges must be made. The front (*M-B′-C′-N*) and breast (*F-L-O-E*) pieces are separate, being securely sewed to the *A-B-C-D* piece along the lines *M-N* and *L-O* respectively. The dimensions of the material required for these two pieces are 4 feet 8 inches by 3 feet 11 inches and 1 foot 11 inches by 3 feet 11 inches respectively. The two separate triangular pieces, *A′-F-Z* and *D′-E-X*, are made from a 3 foot 1½-inch-by 3 foot-10½-inch piece of canvas. This allows for 2-inch laps along the edges. The triangular pieces of canvas

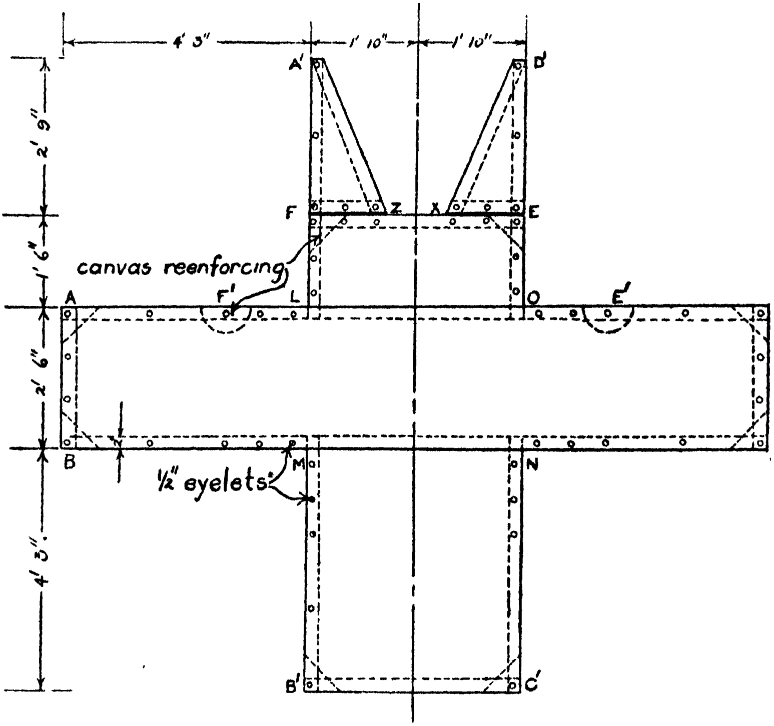

FIG. 3. DIAGRAM OF EXPERIMENTAL HAY BAG

reenforcing shown at the various corners are 4 inches along the right angle sides. The semi-circular pieces of reenforcing at points F' and E' are 3 inches in radius. For further durability, double stitching is recommended for all reenforcing.

The ½-inch eyelets shown in figure 3 are riveted in along the reenforced edges of the bag. The interval between eyelets is 12 to 14 inches in the upper part of the bag and 5 to 6 inches in the lower part. The closer intervals between eyelets in the lower part of the bag are for increased security against the loss of fine roughage particles. Experience has shown that the life of the bag is measured largely by the spacing as well as the firmness with which the eyelets are riveted to the fabric. Each eyelet should be so riveted that the entire eyelet circumference makes firm contact with the material. Also, they should be so spaced that undue stress may not be brought to bear on any single eyelet.

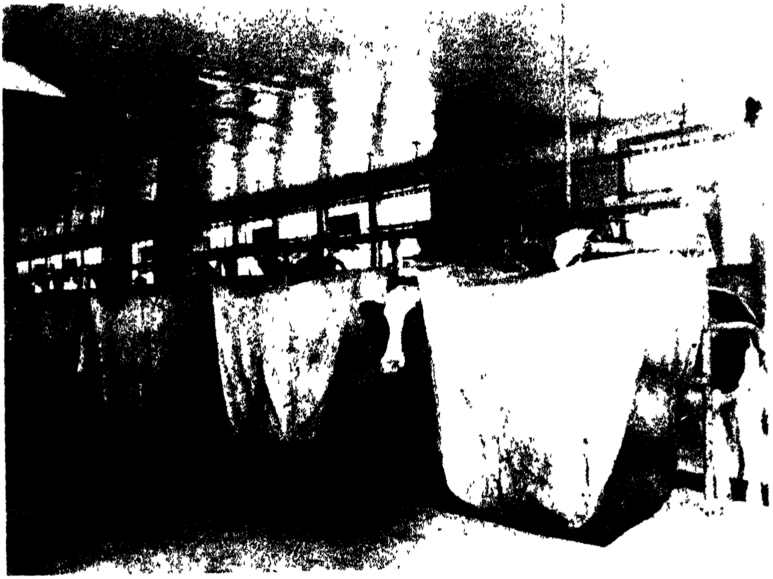

FIG. 4. SHOWING THE HAY FEEDING BAGS IN PLACE AT ALTERNATE STALLS

The dimensions indicated in this paper are for a bag to fit a standard 3-foot-8-inch stanchion. The dimensions must of course be varied to adapt the bag to particular conditions. This bag is made for a manger having a low, rolled front.

This type of bag is not adapted to the feeding of grain and silage because of the deteriorating effects these feeds have upon the bottom of the bag. Consequently, they were devised so that they could be conveniently and quickly removed and returned to place. While the animals were consuming their grain and silage the feeder often weighed the hay allowance, placing it in the respective bags before attaching them to the stalls.

It was found feasible to place the bags before alternate animals, as shown in the photograph, instead of providing a bag for each stall.

FURTHER OBSERVATIONS IN ELIMINATING THE TOXICITY OF COTTONSEED MEAL*

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INTRODUCTION

A previous article in this JOURNAL (1) has called attention to the beneficial effects produced by autoclaving and steaming cotton seeds and cottonseed meal when these products are to be used as livestock feeds. The investigation has been continued and results obtained over longer feeding periods which allowed for observations on the reproduction as well as the growth of animals fed on cottonseed meal diets. Furthermore, the method of procedure has been modified slightly as we have become more familiar with the decomposition products of gossypol, the toxic principle in cotton seeds, and in view of more recent work on the gossypol content of cottonseed meal (2). It seems advisable to report our present results for the benefit of others working along the same lines because these results will be used as a guide in future studies involving considerable outlay and expense as will be necessary in handling large animals. The importance of the work lies in the value of cottonseed meal as a livestock feed which can be enhanced by autoclaving or steaming the meal as previously described (1) (3). We have been able to avert any deleterious effects produced through feeding the meal both to swine, which are very susceptible to injury, and albino rats, which are less so and can consume relatively large amounts without apparent harm. Continued feeding, however, is not desirable.

Believing gossypol to be responsible for the toxicity of cottonseed meal, Sherwood (2) determined by chemical methods the gossypol content of forty samples of cottonseed meal and con-

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cluded that the gossypol content of all but five of the samples was so low that it could not produce injury in albino rats even when the meal constituted as much as 50 per cent of a well-balanced diet. This is based on the assumption that the form of gossypol as ordinarily found in the meal and called d-gossypol is not toxic. This form which is practically insoluble in ether but may be extracted from the meal with hot aniline, will be referred to as d-gossypol in the pages which follow.

Although we have not been able to employ such a large number of meals in our feeding trials, we have determined the gossypol content of many and have been able to estimate their relative toxicities by comparison with a few samples whose toxicities have been determined by chemical and biological methods.

EXPERIMENTAL

Materials and methods

Three samples of cottonseed meal having about the same chemical composition but differing considerably in color and general appearance were selected and fed in adequate diets to albino rats. No attempt was made to use the meal in an otherwise purified ration but rather to feed it in combination with a selected grain, and supply the necessary vitamins as well as increased amounts of sodium chloride and calcium which are usually lacking in grain rations. For our purpose such a procedure was more satisfactory than the use of dextrin and a more complex salt mixture as supplements when the errors which may be introduced in both cases are compared and the results given practical application. Although corn is a common supplement in cottonseed meal rations, some of our previous work has been successfully carried out with wheat and it was therefore chosen as a supplement in these rations.

The different meals made up either 35 or 45 per cent of the rations as shown below, and were employed in the larger amounts to make more pronounced the effects of the feeding. We have at times, employed smaller amounts of different meals of unknown gossypol content and obtained similar conditions in animals as are reported here, but in such cases other factors are involved which complicate the interpretation of results. The rations employed in this study were made up as follows:

Cottonseed meal	35–45
Wheat	60–50
$CaCO_3$	1– 1
NaCl	1– 1
Cod liver oil	3– 3

The gossypol and d-gossypol contents of the meals are given together with other information in table 1. The gossypol was determined by Carruth's method as modified by Schwartze and Alsberg (4) for cotton seeds and the so-called d-gossypol or that portion which is quite insoluble in oil and ether was determined by the method used by Sherwood (2) using petrolic ether for washing the precipitates.

TABLE 1

Data on the cottonseed meals

NUMBER	TREATMENT	COLOR	GOSSYPOL	D-GOSSYPOL	RATIONS IN WHICH USED
			per cent	*per cent*	
XX	As bought	Bright yellow	—	0.535	XX
XX	Extracted	Light yellow	—	0.535	XXII
XX	Autoclaved	Brown	—	0.326	XXI
XXV	As bought	Yellow brown	Trace	0.918	XXV
XXV	Extracted	Light yellow	—	0.918	XXVI, XXIV
XXV	Autoclaved	Brown	—	0.547	XXVII, XXIII
XXX	As bought	Light yellow	Trace	0.756	XXX, XXXI
XXX	Autoclaved	Brown	—	0.398	XXXII

As noted in the table, the rations were made up with "raw" meal or as bought on the market, ether-extracted, and autoclaved.

The extraction of the meal with ether was accomplished for the purpose of removing that portion of the gossypol which is ether-soluble and is in the same form as it is found in the seeds, in which form it is extremely toxic. The oil so removed was replaced with refined cottonseed oil, which is free of gossypol, such that when 35 per cent extracted meal was used in a ration there was in reality only about 32 per cent extracted meal plus

3 per cent refined oil. In this way the oil content as well as the total cottonseed meal content of the rations was regulated and the rations made comparable to each other. Any toxicity noted with the use of extracted meal then, would be due to this insoluble form of gossypol, which has been suggested as being non-toxic (2).

Autoclaving the meals in the presence of excess moisture at 20 pounds pressure as described elsewhere (1) was for the purpose of destroying both forms of gossypol, and when fed, served as a control as well as an experimental ration when compared to other feeding rations not recorded here. It is significant to notice that this process did not entirely destroy the insoluble gossypol in all cases although it did decrease the original amount to a varying degree in each meal. These variations and the more complete destruction of gossypol in meal XXX are explained as being due to factors difficult to control during the autoclaving, chief of which is the packing of the meal which is unfavorable to the action of the steam. The consistency of meal XXX quite different from the others, was such that it remained open and porous during this time with the result that the steam more readily penetrated the entire mass and a product containing much less d-gossypol was produced. These difficulties were not encountered when autoclaving cotton seeds.

RESULTS

To eliminate numerous tables, the data obtained and the general results of the feeding work with the rations employed are tabulated in table 2.

DISCUSSION OF RESULTS

A comparison of the growths of the animals receiving the different meals similarly prepared reveals the fact that the effects of the meal are not always evident by observing the growth rates of the animals but is oftentimes apparent in the number of breeding failures as determined by the number of litters born. Furthermore, death sometimes resulted among those animals showing normal or slightly subnormal growth while some other animals lived through a longer period, but made smaller gains. These conditions are considered in what follows. In all cases the autoclaved meal was superior for both growth and reproduction and although the chemical determinations showed it to contain some gossypol compound due perhaps to incomplete autoclaving, the presence of this compound in these amounts did not appear to be detrimental. Whether or not the forms of gossypol as found in the autoclaved and the ether-extracted meal

TABLE 2

Results of cottonseed meal feeding

RATION	COTTONSEED MEAL	FORM OF MEAL	GOSSYPOL IN RATION	D-GOSSYPOL IN RATION	NUMBER OF ANIMALS	NUMBER OF FEMALES	DURATION OF EXPERIMENT	GROWTH	LIVED	DIED	NUMBER OF LITTERS	TOTAL YOUNG	YOUNG LIVED	YOUNG DIED
Cottonseed meal XX														
	per cent		*per cent*	*per cent*			*mos.*							
XX	35	"Raw"	—	0 187	4	2	6	+++	1	3	1	10	—	10*
XXI	35	Autoclaved	—	0 114	4	2	6	+++	3	1	3	20	18	2
XXII	35	Extracted	—	0 187	4	2	6	+++	—	4	—	—	—	—
Cottonseed meal XXV														
XXV	45	"Raw"	Trace	0 413	4	2	5	+	4	—	2	3	2	1
XXV	45	"Raw"	Trace	0 413	3	2	2	+	3	—	—	—	—	—
XXVI	45	Extracted	—	0 413	4	2	5	++	4	—	3	11	8	3
XXIII	35	Extracted	—	0 321	3	1	6	+++	2	1	2	14	4	10†
XXVII	45	Autoclaved	—	0 246	4	2	5	+++	4	—	5	31	25	6
XXIV	35	Autoclaved	—	0 191	3	2	6	+++	3	—	4	24	20	4
Cottonseed meal XXX														
XXX	35	"Raw"	Trace	0 264	4	2	5	++	2	2	2	20	20	—‡
XXXI	45	"Raw"	Trace	0 340	4	2	5	+	4	—	—	—	—	—
XXXII	45	Autoclaved	—	0.179	4	2	5	+++	4	—	4	26	17	9§
XXXII	45	Autoclaved	—	0 179	2	1	2	+++	2	—	—	—	—	—

* Mother died ten days after young born.

† One month old at time of death.

‡ Born fifteen days before experiment ended.

§ Mothers did not take care of first litters born.

are the same and equally toxic is left undecided. Further study of these decomposition products of gossypol is needed.

Cottonseed meal XX

This meal in which no gossypol was found by chemical determinations but showed a d-gossypol content of 0.535 per cent was fed during the summer months. The animals died several days apart during a spell of hot weather. Just previous to their deaths which were quite sudden, some of the animals appeared suffocated, running about the cage and breathing with difficulty, and dying soon afterwards. It is noteworthy that a similar condition has been observed among larger animals fed on heavy cottonseed meal rations and that previous investigation in this laboratory has disclosed the fact that gossypol lowers the oxygen carrying capacity of the blood (5). Furthermore, it is a common practice among feeders to reduce the amount of cottonseed meal in animal rations during the summer months. Extreme cold appeared to increase the effects of gossypol in studies conducted by Schwartze and Alsberg (6). In face of these facts we believe that the deaths of our animals were due primarily to the cottonseed meal diets and were hastened by the extreme temperature change. Further evidence of this lies in the fact that none of the rats in the stock colony and only one of the rats receiving the autoclaved meal rations suffered the same consequences. The early deaths of the animals prevented conclusive results as to reproduction except as those animals receiving the autoclaved meal reproduced sooner and showed no abnormalities in the third generation. In no case have rats been raised through three generations on either the "raw" or extracted meals. Extraction of the meal was of little if any help in eliminating its toxicity.

Cottonseed meal XXV

This meal which showed only a trace of gossypol and 0.918 per cent d-gossypol was used in the same and larger amounts. Its effects when compared to the same meal which had been autoclaved were manifested by slow growth and poor reproduction, although no deaths resulted. Extraction increased the value of the meal for growth and reproduction when the meal was used in the larger amounts, although the young reared did not make normal gains after weaning. When used in the smaller amounts as in ration XXIV, growth was normal with a high death rate among the young.

Cottonseed meal XXX

This meal which also showed a trace of gossypol and a d-gossypol content of 0.756 per cent proved to be the most toxic of the three. Such would be indicated from the color of the meal since the heat treatment at the oil mill if of short duration tends to destroy less gossypol and produce a lighter colored meal.

Two of the animals receiving 35 per cent "raw" meal died after making fair growth, and only those receiving the autoclaved meal grew and reproduced satisfactorily.

Increased amounts of the "raw" meal decreased the growth rate without producing any deaths but there was no reproduction. The rapid decline of these animals indicates the presence of greater quantities of gossypol in the meal than were shown by the chemical determinations.

Of the 19 rats receiving in their ration 35 to 45 per cent "raw" cottonseed meal over a period of two to six months, only 4 showed normal growth and 5 died as a result of the feeding. Among the 11 rats receiving the same amounts of meal extracted with ether, there were 5 deaths and the growth rates showed a slight increase. Of the 17 rats receiving the autoclaved meal, only one died and all made normal growth, rearing their young in several instances through two generations.

Reproduction was near normal only in the case of those animals fed the autoclaved meal, the 8 females in this group giving birth to 101 young as compared to 33 young born to the same number of females receiving the "raw" meal and 25 young from 5 females receiving the extracted meal.

SUMMARY

A study was made of the effects of feeding cottonseed meal rations to experimental animals when the meal constituted from 35 to 45 per cent of the diet. Growth and reproduction records of the animals were obtained over a period of five to six months. Three different cottonseed meals whose gossypol and d-gossypol contents had been estimated by chemical methods were employed and fed in three different forms; "raw" or as bought on the market, ether-extracted, and autoclaved. The meals were prepared in these forms for the purpose of altering their gossypol contents.

The "raw" meals, which probably contained some "free" gossypol in the same form as it is found in the seeds although only traces could be detected by chemical methods, had a pronounced toxic effect as revealed in the slow growth of the animals and poor reproduction.

Ether extraction of the meal which removes some of the gossypol in a proven toxic form, aided but little in preventing these injurious effects indicating that the insoluble form of gossypol which occurs in relatively large amounts is also toxic.

Autoclaving the moist meal which reduces its toxicity but did not entirely destroy the insoluble gossypol produced a feed which when properly supplemented afforded good growth and reproduction.

These results although perhaps not directly applicable, have an important bearing upon the feeding value and methods of preparation of cottonseed meal for all types of animals.

In conclusion the author makes grateful acknowledgment to Dr. V. G. Heller and Dr. N. B. Guerrant for their constant interest and aid during the progress of these investigations with cottonseed meal.

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PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

HELD AT EAST LANSING, MICHIGAN, JUNE 22, 23 AND 24, 1927

The summer meeting was held as planned at the Michigan Agricultural College June 22, 23 and 24. The program was arranged by the committee consisting of C. H. Eckles of Minnesota, chairman, H. A. Ruehe of Illinois, and C. C. Hayden of the Ohio Experiment Station.

The meeting was opened at 9:30, June 22 with Professor O. E. Reed of Michigan presiding. After a few remarks he introduced Dean Shaw who pointed out that the Michigan Agricultural College, established in 1857, was the first agricultural college in America. Since its establishment there has been added curricula courses in engineering, home economics, forestry, veterinary science and liberal arts in the order named. Dean Shaw mentioned that industrial wages in Michigan amounted to eight hundred million dollars a year, half of which goes for the products of the farms. Professor Reed then introduced Dean Phelan who gave a general talk on vocational teaching. President Fitch who had arrived now took the chair and introduced Professor Sherman who emphasized the fundamentals in the curriculum for dairy manufacturing students and also recommended the consolidation of courses in technical dairy subjects. The discussion was entered into by Eckles, Savage, Reed, Regan, Guthrie and Davis.

The afternoon session went off as scheduled with much interest in the papers and considerable discussion following each. The tour of the barns and explanation by Reed and Huffman of the voluminous mineral feeding trials in progress attracted much interest.

On this same evening the staff of the Dairy Department of the Michigan Agricultural College entertained the visitors at the Woman's Building from 8 to 10.

Following the social entertainment there was a meeting of the executive council of the association at which there were present: Fitch, Sherman, Guthrie, Frandsen, Eckles, Cramer, Reed, Ragsdale, Cave, Ruehe and White. There had been a desire expressed by a number to complete the program on Friday earlier than the scheduled time. To this end the executive committee voted to begin the morning session on Friday at 9:00, to condense the time allotted to the discussion on extension problems and begin the section sessions at 10:30 instead of 1:30 in the afternoon. This was presented at the convention on Thursday morning and adopted unanimously.

As there were expressions of general satisfaction with the attendance and the program the executive committee discussed the matter of proclaiming this the annual meeting and of turning over the meeting at the National Dairy Show entirely to the Southern Division. After considerable discussion it was voted that the officers should plan for a summer meeting in 1928 as the annual meeting and that the present meeting be declared the annual meeting for 1927. This action was approved on Thursday morning by the convention.

On Thursday Vice President Sherman presided.

President Fitch appointed the following nominating committee: W. M. Regan, Chairman, H. A. Ruehe, A. J. Cramer, C. E. Wylie and E. S. Guthrie. In the afternoon this committee made the following report which was adopted. For President, G. C. White, A. C. Ragsdale; for Vice President, A. C. Baer, W. J. Fraser; for Secretary-Treasurer, J. M. Sherman, R. B. Stoltz; for Editor of the JOURNAL OF DAIRY SCIENCE, O. A. Dahlberg, J. H. Frandsen.

All the papers on Thursday proved of vital interest and created as much discussion as the brief time would permit. Dr. Hood, Chief of the Division of Dairy Research, Dairy and Cold Storage Branch at Ottawa, Canada, was called upon and spoke briefly with reference to the quality and improvement control work that has recently been undertaken with Canadian export butter.

Directly following this, the executive committee held a brief session. There were present at this session: Fitch, Cramer, Frandsen, Ragsdale, Guthrie, Sherman and White. It was the sense of this meeting that the sections could elect their officers during the present sessions or at Memphis according to the time that seemed best to meet the convenience of the particular sections. The manufacturer's section elected the following officers: Dr. W. V. Price, Chairman; and Professor P. S. Lucas, Secretary. The other sections decided to elect officers for 1928 at the National Dairy Show at Memphis in October. The committee had invitations before it for the 1928 meeting from the Ohio State University, University of Illinois, Cornell University, University of Minnesota and University of Wisconsin.

On Thursday evening a committee on entertainment of the Michigan Agricultural College Dairy Department furnished transportation out to Palmer Lake where a very satisfactory fish dinner was enjoyed followed by dancing at the lake pavilion.

The Friday session was called to order by Mr. Cramer at 9:00 o'clock. The Extension Conference held in Madison, Wisconsin in May was reported upon by A. C. Baltzer. R. H. Addy was called upon by the Chairman to explain some charts with reference to the extension work in the state of Michigan. O. E. Reed then spoke and he made a plea that very earnest thought be given to the development of plans vital to the improvement of dairy herds. He pointed out that advanced registry testing was not gaining ground and that the average breeder was today much more concerned with records made under herd conditions than he was in world records. Reed pointed out that there is much interest in some sort of a herd test plan by all the breed associations. As is generally known, the Ayrshire Association has a herd test plan already in use and the Holstein Association at its June meeting appointed a committee, on which there are a number of Dairy Science Association members, to give thorough consideration to some type of herd test plan for the Holstein breeders. It is evident that this gives the Dairy Science Association an opportunity to give thorough consideration to this type of test and it is hoped that by its influence a uniform system of testing can be introduced into each of the breeds when they want to adopt a plan of this character.

Eckles agreed that the cow test association idea does not completely imply the significance of this type of work in herd improvement and suggested that the name of these organizations be changed to Dairy Herd Improvement Associations to better represent the character and purpose of cow testing work. This proposal was seconded and voted after discussion by Ragsdale and Yapp.

After the discussion by Potts and Beuchel of the Bureau of Agricultural Economics, U. S. D. A., it was voted that it was the sense of the meeting that the executive committee should authorize the organization of a section on Dairy Economics as soon as the necessary steps had been taken to comply with the constitutional requirements.

It was moved by Ragsdale and voted that the Secretary be instructed to express the appreciation of the members to Dean Shaw of the Michigan Agricultural College and to Professor Reed and his staff for their efforts in making the meetings such a marked success.

The complete program follows.

G. C. WHITE, *Secretary.*

PROGRAM*

THE TEACHING OF DAIRYING

President J. B. Fitch presiding

Opening Remarks	Dean R. S. Shaw
Announcements	Pres. J. B. Fitch
Vocational teaching	Dean Phelan, Education Dept., Mich. Agr. College
Importance of the fundamentals in dairy instruction	J. M. Sherman, Cornell Univ.
Teaching cattle breeding	W. W. Yapp, University of Illinois
Fitting our curriculum to meet the demands of industry	H. A. Ruehe, University of Illinois
The first course in dairying. What it should contain and how it should be taught.	H. P. Davis, University of Nebraska
Demonstration of experimental work in progress at Michigan Experiment Station	O. E. Reed, C. F. Huffman

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* Some of the papers and several abstracts of papers delivered at this meeting will appear in the Journal from time to time.—EDITOR.

RESEARCH PROGRAM

J. M. Sherman, Vice-President, presiding

The possibility of producing iodized milk; a progress report of the study of the iodine content of milk as affected by feeding iodine
C. F. Monroe, Ohio Exp. Station
The effect of high and low protein diets on growth and development of the internal organs............................S. W. Mead, Univ. of California
High and low protein rations ....A. E. Perkins, Ohio Exp. Station
The economic effect of contagious abortion
R. F. Morgan, University of Nebraska
Chemical sterilization in the dairy industry
M. J. Prucha, J. M. Brannon, Univ. of Ill.
The food value of milk as affected by rations. The effect of widely differing rations on the vitamin content of milk..W. E. Krauss, Ohio Exp. Station
The adaptation of the MacDonald process for the removal of onion odor and flavor in milk in creamery practice....C. E. Wylie, Univ. of Tennessee
Recent results concerning Vitamin B requirements for calves
S. I. Bechdel, State College, Pa.
Recent development in calcium and phosphorus metabolism
C. F. Huffman, Mich. Exp. Station
The effect of butterfat on overrun and quality
P. S. Lucas, Michigan Exp. Station
Sugar in ice cream...... ....W. H. Martin, Kansas Exp. Station
Research problems in dairy production on the Pacific coast
W. M. Regan, Univ. of California
Effect on the ice cream mix of adding gelatin and sugar at different times and of homogenizing before and after condensing
J. C. Hening, Geneva, N. Y. Exp. Station
Studies in the manufacture of cream and Neufchatel cheese
J. C. Marquardt, Geneva, N. Y. Exp. Station
The relation between pH and titratable acidity in milk
P. F. Sharp and T. J. McInerney, Cornell Univ.
Butter vs. oleo in the ricket control in pigs..E. L. Anthony, W. Va. Exp. Station
A statistical study of the Babcock test..D. H. Nelson, California Exp. Station
A successful attempt to improve the quality of creamery butter
J. M. Thurston, Univ. of Minnesota
Sorghum grains for growth and for milk production
H. W. Cave, Kansas Exp. Station

EXTENSION SECTION

A. J. Cramer, Chairman Extension Section, presiding

Report of the extension conference at Madison..O. E. Reed and A. C. Baltzar
General discussion of extension problems.
Reports from Bureau of Agr. Ex. Section of Marketing.

JOINT MEETING PRODUCTION, EXTENSION AND OFFICIAL TESTING SECTIONS

A. C. Ragsdale, Chairman Production Section, presiding

Feeds and feeding laboratory..................E. S. Savage, Cornell University
Teaching dairy cattle judging..............O. G. Schaefer, Univ. of Minnesota
College Feed Conference Board and open formulae feeds
A. R. Merrill, Conn. Agri. College
Present day problem in official testing........................General discussion

MANUFACTURERS' SECTION

E. S. Guthrie, Chairman Manufacturers' Section, presiding

Physical factors influencing the formation and fat content of cream separated from milk by gravity..H. C. Troy and P. F. Sharp, Cornell University
The oxidation of fat in relation to the keeping qualities of dairy products
G. E. Holm, U. S. D. A.
The comparison of the methylene blue reductase test with bacterial counts, acidity and sediment tests..................A. S. Ambrose, Univ. of Illinois
Composition and shrinkage of creamery print butter in relation to working
V. C. Manhart, Purdue University
Factors affecting the whipping qualities of ice cream mixes
H. H. Sommer, University of Wisconsin
Hydrogen-ion concentration in cold storage butter
E. H. Parfitt, Purdue University
The effect of freezing milk before and subsequent to pasteurization on the physical properties of milk..........W. H. E. Reid, University of Missouri
The relation of acidity of cream to the quality of butter with special consideration for variations in the acidity below and above 0.3 per cent
W. White, U. S. D. A.
The development of dairying in the Pacific Northwest
J. A. Nelson, Montana Exp. Station

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